V. Developmental and Reproductive Effects

The Center for the Evaluation of Risks to Human Reproduction (CERHR) Expert Panel on DINP (CERHR 2000a) and the CHAP (CPSC 2001) have reviewed the reproductive and developmental effects of DINP in detail. Data on these effects are summarized below.

A. Developmental Toxicity

Two published prenatal developmental toxicity studies in rats were available for DINP (Hellwig et al. 1997; Waterman et al. 1999). In both studies, dams were dosed by gavage on gestational day (GD) 6–15 and sacrificed on GD 20–21. Developmental toxicity was also noted in a one-generation and a two-generation reproductive study.

Hellwig et al. (1997) studied the developmental effects of three types of DINP (DINP-1, DINP-2, DINP-3). Wistar rats (10/group) were given doses of 0, 40, 200, and 1,000 mg/kg-d (Table V-1). Relative kidney and liver weights were slightly increased in dams of the highest dose group (5–13%), but in most instances were not statistically significant. Fetal viability and body weight were unaffected in all three studies. The incidences of skeletal variations (rudimentary cervical ribs, accessory 14^{th} ribs) were elevated, with the number of affected fetuses per litter being significantly higher than controls with DINP-1 and DINP-3. There also were increases in dilated renal pelves (DINP-1 and DINP-3), agenesis of kidneys and ureters (DINP-3), hydroureter (DINP-3), and skeletal (shortened and bent humerus and femur) malformations (DINP-3) in the high-dose group. The CERHR Expert Panel identified a NOAEL of 200 mg/kg-d and a LOAEL of 1,000 mg/kg-d for maternal and developmental effects in the Hellwig et al. study.

Waterman et al. (1999) treated Sprague-Dawley rats (25/group) with DINP-1 at doses of 0, 100, 500, or 1,000 mg/kg-d. Maternal toxicity at the highest dose was evidenced by decreased food intake and weight gain. The authors concluded that the LOAEL for maternal and developmental toxicity was 1,000 mg/kg-d, with a NOAEL of 500 mg/kg-d. However, the Expert Panel concluded that developmental effects were present at 500 mg/kg-d (CERHR 2000a). At the request of the CERHR Panel, the study sponsor reanalyzed the data, the results of which supported the Expert Panel's conclusion. Thus, the Expert Panel determined that the NOAEL for the study was 100 mg/kg-d.

Gray et al. (2000) studied the effects of perinatal exposure to DINP and other phthalates on sexual differentiation in male Sprague-Dawley rats. Dams were given 750 mg/kg phthalate from GD 14 to postnal day (PND 3). Male pups exposed to DINP exhibited female-like areolas/nipples (22%, $p<0.01$) and reproductive malformations (7.7%, $p<0.04$). The frequencies of malformations were greater with DEHP—areolas/nipples (87%) and reproductive organ malformations (82%). Malformations associated with DINP exposure included: small or atrophic testes; flacid, fluid-filled testes, epididymal agenesis with hypospermatogenesis, and scrotal fluid-filled testis devoid of spermatids. Dose-dependent increases in the incidence of areolas and reduced anogenital distance in

males were observed in a subsequent study at 1,000 and 1,500 mg/kg-d (Ostby et al. 2001).

Adverse effects on weight gain in pups during the perinatal and pre-weaning period of life were evident in a two-generation reproductive study with Sprague-Dqwley rats (Waterman et al. 2000). Dams were fed 0, 0.2, 0.4, or 0.8 percent DINP during mating, gestation, and lactation. Certain effects of male reproductive tract development, identified as a sensitive target with other phthalates (compare Gray et al. 2000), were not examined. In the F_1 generation, pup weights were significantly reduced in males on postnatal day (PND) 0 at 0.8 percent DINP and in females at 0.4 and 0.8 percent DINP (PND 7, 14). By PND 21, pup weights were reduced at doses ≥0.2 percent DINP. In the F_2 generation, male pup weights were reduced at 0.4 percent DINP (PND 7, 14, 21) and in females at 0.2 percent (PND 4, 7, 14, 21). The Expert Panel identified a LOAEL for developmental effects of 0.2% (143–285 mg/kg-d during gestation through lactation).

Developmental effects of two 2 isononyl alcohols were studied in Wistar rats (Hellwig and Jackh 1997). Toxicity was more severe with type 1 isononyl alcohol, the alcohol used to manufacture DINP-1. Maternal mortality was observed at 1,440 mg/kg-d with type 2 and at doses ≥1,080 mg/kg-d with type 1. Fetal malformations and/or variations occurred at ≥1,080 mg/kg-d. Increases in skeletal variations and retardations were observed with type 1 at 720 mg/kg-d. The NOAEL for the study was 144 mg/kg-d.

Phthalate esters are believed to induce developmental effects in males by decreasing testosterone levels during sexual differentiation (Parks et al. 2000). The ability of phthalates to cause maternal and developmental effects is independent of the PPARα receptor (Peters et al. 1997c; see also Ward et al. 1998). Phthalate esters have been shown to induce developmental effects in several species, including rabbits, guinea pigs, and hamsters (as cited in Parks et al. 2000).

B. Reproductive Toxicity

Reproductive effects were studied in one- and two-generation feeding studies in rats (Waterman et al. 2000; reviewed in CERHR 2000a and CPSC 2001). Rats were administered dietary levels of 0, 0.5, 1.0, or 1.5% DINP in the one-generation dose range finding study, and levels of 0, 0.2, 0.4, or 0.8% in the two-generation study (Table V-1). In the two-generation study, reproductive parameters including mating, fertility, and testicular histology were unaffected in both generations at the high dose (0.8%; 665–779 and 696–802 mg/kg-d in males and females, respectively). Developmental effects including decreased pup weight gain were observed (discussed above). Histologic effects included mild hepatic eosinophilia in both sexes of parental rats in all dose groups of both generations and dilated renal pelves in F_1 parental males at 0.4 and 0.8 percent DINP. In the one-generation study, fertility was unaffected in male and female rats exposed to dietary DINP concentrations as high as 1.5% (966–1,676 and 1,114–1,694 mg/kg-d in males and females, respectively). The CERHR concluded that male and female rat reproductive function and structure of reproductive organs are unaffected by exposure to DINP at maternal doses of 555–1,129 mg/kg-d during gestation and lactation,

respectively, and adult doses as high as 1,676 mg mg/kg-d in males and 1,694 mg/kg-d in females.

C. Summary of Reproductive and Developmental Toxicity

The CPSC staff concludes that there is sufficient evidence that prenatal exposure to DINP causes developmental effects in experimental animals. Therefore, DINP is considered to be a probable developmental toxicant in humans, based on sufficient evidence in experimental animals (CPSC 1992). The lowest NOAEL for these effects is 100 mg/kg-d. However, because systemic health effects represent a more sensitive endpoint (NOAEL=15 mg/kg-d), the latter endpoint will be used for quantitative risk assessment.

Table V-1. Reproductive and developmental effects of DINP

Study	Doses, species/strain	NOAEL [a] mg/kg-d	LOAEL mg/kg-d	Effects
Hellwig et al. 1997	0, 40, 200, 1000 mg/kg-d (GD 6-15) Wistar rat	200	1000	Maternal effects (increased kidney & liver weight)
		200	1000	Dilated renal pelves, hydroureter; skeletal variations & malformations
Gray et al. 2000	0, 750 mg/kg-d (GD 14 – PND 3) SD rat	ND	750	In males only: areolas/nipples; reproductive malformations
Ostby et al. 2001	0, 1000, 1500 mg/kg-d (GD 14 – PND 3) SD rat	ND	1000	In males only: areolas; reduced anogenital distance
Waterman et al. 1999	0, 100, 500, 1000 mg/kg-d (GD 6-15) SD rat	500	1000	Maternal effects (decreased weight gain)
		100	500	Dilated renal pelves; lumbar ribs; cervical ribs
Waterman et al. 2000	0, 0.2, 0.4, 0.8% in feed (2-generation study) SD rat	ND	143-285 (0.2%)	Decreased pup weight
		ND	165-189	Histologic effects (hepatic eosinophilia, dilated renal pelves) (parental)
		665-779 (0.8%)	ND	Mating, fertility, testicular histology (males)
		696-802 (0.8%)	ND	Mating, fertility (females)

[a] GD, gestational day; LOAEL, lowest observed adverse effect level; ND, not determined; NOAEL, no observed adverse effect level; PND, postnatal day.

VI. Genotoxicity

Although peroxisome proliferators are believed to induce oxidative stress, they generally exhibit little or no evidence of genotoxicity in standard assays (Galloway et al. 2000). DINP was not mutagenic in *Salmonella* (BASF 1986, 1995; EG&G Mason Research 1980; Exxon, 1996a; McKee et al. 2000; Zeiger et al. 1985) or mouse lymphoma cells (Barber et al. 2000; Cifone, 1986). DINP did not induce unscheduled DNA repair in primary rat hepatocytes (Litton Bionetics 1981a). It failed to induce phenotypic transformation of BALB/C-3T3 mouse cells in one experiment with metabolic activation (Barber et al. 2000; Microbiological Associates, 1981a) and in five experiments without activation (Barber et al. 2000; Litton Bionetics 1981b,c; Litton Bionetics 1985; Microbiological Associates 1981b,c), although one such experiment without metabolic activation gave a small, but statistically significant positive effect (Microbiological Associates, 1981d). In addition, DINP tested negative in various *in vitro* (Exxon 1996b; McKee et al. 2000) and *in vivo* (McKee et al. 2000; Microbiological Associates, 1981e) chromosome damage assays.

VII. Carcinogenicity

This section reviews the evidence for the carcinogenicity of DINP in rats and mice, and mechanistic data, such as peroxisome proliferation. Carcinogenic mechanisms and their relevance to human risk are also discussed. The primary types of neoplasms induced in rodents by DINP were hepatocelluar adenoma and carcinoma in rats and mice, renal tubular cell tumors in male rats, and mononuclear cell leukemia in Fischer rats. Carcinogenicity data and mechanisms were reviewed in detail by the CHAP (CPSC 2001).

The carcinogenicity of DINP has been tested in four 2-year feeding studies in rats and mice (Table VII-1). DINP-1 was studied in Fischer 344 rats at doses of 0, 0.03, 0.3, and 0.6 percent in feed in a study by Lington et al. (1997). Interim sacrifices were done at 6, 12, and 18 months. DINP-1 from a different supplier was tested in Fischer 344 rats at doses of 0, 0.05, 0.15, 0.6, and 1.2 percent in feed (Moore 1998a) and in B6C3F1 mice at doses of 0, 0.05, 0.15, 0.4, and 0.8 percent (Moore 1998b). The studies by Moore are also referred to as the Covance studies (CPSC 1998; CPSC 2001). Both Covance studies included interim sacrifices at 1, 2, 13, and 79 weeks and recovery groups exposed for 78 weeks at the high dose, followed by a 26-week recovery period. DINP-A (71549-78-5), which is believed to be similar to DINP-2, was tested in Sprague-Dawley CD rats at doses of 0, 0.05, 0.5, and 1.0 percent in feed (Bio/dynamics 1986). An interim sacrifice was performed at one year.

Table VII-1. Lifetime dietary studies of DINP

Study	Test material	Species, strain	Doses	Number per dose/sex group
Lington et al. 1997	DINP-1	F344 rat	0, 0.03, 0.3, 0.6 % in feed (M: 0, 15, 152, 307 mg/kg-d F: 0, 18, 184, 375 mg/kg-d)	~80 per group
Moore 1998a (Covance)	DINP-1	F344 rat	0, 0.05, 0.15, 0.6, 1.2 % in feed [a] (M: 0, 29, 88, 359, 733 mg/kg-d F: 0, 36, 109, 442, 885 mg/kg-d)	65, 50, 50, 65, 65
Bio/dynamics 1986	DINP-A	SD rat	0, 0.05, 0.5, 1.0 % in feed (M: 0. 27, 271, 553 mg/kg-d F: 0, 33, 331, 672 mg/kg-d)	70 per group
Moore 1998b (Covance)	DINP-1	B6C3F1 mouse	0, 0.05, 0.15, 0.4, 0.8 % in feed [a] (M: 0, 90, 276, 742, 1560 mg/kg-d) F: 0, 112, 336, 910, 1888 mg/kg-d)	70 per group

[a] Includes a recovery group in which animals were exposed at the high dose for 78 weeks, followed by a 26 week recovery period.

A. Liver

1. Incidence Data

a. Rats

Lington treated Fischer 344 rats with up to 0.6 percent DINP-1 in feed (Lington et al. 1997). Incidences of hepatocellular carcinoma and neoplastic nodules were reported. There was a small, non-significant increase in the incidence of hepatocellular carcinoma in males at the high dose (0.6%) ($p=0.12$), although the trend test was positive ($p=0.015$) (Table VII-2). Lington et al. also found an increased incidence of a slight centrilobular to midzonal hepatocellular enlargement at the high dose in both sexes, which may be related to peroxisome proliferation (Table IV-2).

In the Covance study, Fischer rats were given dietary doses of up to 1.2 percent DINP (Moore 1998a). The overall incidence of hepatocellular carcinoma ($p<0.001$) was significantly elevated in males at the high dose (1.2%), as was the incidence of carcinoma or adenoma ($p<0.001$) (Table VII-3). In females, the incidence of carcinoma or adenoma was significantly elevated ($p=0.017$), while carcinoma alone was slightly elevated ($p=0.097$). With males and females, the tumor incidences at 79 weeks were at or near background levels. In addition, the incidences of carcinoma in the recovery groups were not significantly different from the controls, which is consistent with the observation that most tumors appeared after 79 weeks. Hepatocelluar proliferation was increased at one week, but not at 2, 13, or 104 weeks, suggesting that DINP induces acute phase, but not chronic, hepatocellular proliferation. Palmitoyl-CoA oxidase activity (an indicator of peroxisome proliferation) and hepatocellular enlargement were elevated at the high dose in males and females.

In the Bio/dynamics study with DINP-A, rats were given dietary doses up to 1.0 percent (Bio/dynamics 1986). The incidence of hepatocellular carcinoma was significantly elevated in females at the mid ($p=0.029$) and high ($p=0.007$) doses (Table VII-4). There was a small, but non-significant increase in males.

b. Mice

In B6C3F1 mice, benign and malignant liver tumors were elevated in both sexes at the two highest doses (0.4 and 0.8%) (Moore 1998b). In males, the incidence of hepatocellular carcinoma was significantly elevated at 0.8 percent DINP ($p=0.017$), while the incidence of carcinoma or adenoma was significantly elevated at 0.4 ($p=0.008$) and 0.8 percent (Table VII-5). In females, the incidence of carcinoma was significantly elevated at both 0.4 and 0.8 percent, while the combined incidence of carcinoma and adenoma was significantly elevated at doses of 0.15 percent and greater. In males and females, the tumor incidences were generally close to background at 79 weeks. In males, the incidence of carcinoma in the recovery group was not significantly different from the control. In females, however, the incidence of carcinoma in the recovery group remained significantly elevated ($p=3.8\times10^{-5}$ by Fisher exact).

Table VII-2. Incidence of hepatocellular neoplasia in a 2-year dietary study of DINP-1 in Fischer 344 rats (Lington et al., 1997) [a, b]

Lesion	Percent DINP in feed			
	0	0.03	0.3	0.6
Males				
Carcinoma [b]	0/81	0/80	0/80	3/80 [c]
Neoplastic nodules or carcinoma	3/81	1/80	1/80	4/80
Females				
Carcinoma	1/81	0/81	0/80	1/80
Neoplastic nodules or carcinoma	1/81	2/81	0/80	2/80

[a] Adapted from CPSC 2001
[b] p=0.015 for Fisher's exact trend test.
[c] p=0.12 for Fisher exact test for pairwise comparison with control.

Table VII-3. Incidence of hepatocellular neoplasia in a 2-year dietary study of DINP-1 in Fischer 344 rats (Moore, 1998a) [a]

Lesion	Percent DINP in feed					
	0	0.05	0.15	0.6	1.2	1.2 R[b]
Males						
Carcinoma						
Overall incidence	1/65	0/50	0/50	1/65	12/65	2/50 [e]
At 79 weeks	0/10	NA	NA	0/10	1/10	(p=0.40)
Poly 3 [c, d]	p<0.001	–	–	–	p<0.001	
Carcinoma or adenoma						
Overall incidence	5/65	3/50	2/50	7/65	18/65	–
At 79 weeks	1/10	NA	NA	0/10	1/10	
Poly 3	p<0.001	–	–	–	p<0.001	
Females						
Carcinoma						
Overall incidence	1/65	0/49	0/50	1/65	5/65	2/55 [e]
At 79 weeks	0/10	NA	NA	0/10	0/10	(p=0.44)
Poly 3	p=0.002	–	–	–	p=0.097	
Carcinoma or adenoma						
Overall incidence	1/65	1/49	0/50	2/65	8/65	–
At 79 weeks	0/10	NA	NA	0/10	1/10	
Poly 3	p<0.001	–	–	–	p=0.017	

[a] Adapted from CPSC 2001.
[b] Recovery group. Animals were exposed for 78 weeks, followed by a 26-week recovery period.
[c] Significance value for trend is given in the column for the control group.
[d] Statistical analysis was provided by the National Toxicology Program. Additional statistical tests are found in CPSC 2001, Appendix B, part A.
[e] Level of significance for the recovery group computed by Fisher Exact test. (Statistical tests were not run by NTP on the recovery group.)

Table VII-4. Incidence of hepatocellular neoplasia in a 2-year dietary study of DINP-A in Sprague-Dawley rats (Bio/dynamics, 1986) [a, b]

Lesion	Percent DINP-A in feed			
	0	0.05	0.5	1.0
Males				
Neoplastic nodules	2/70 (2.9%)	5/69 (7.2%)	6/69 (8.7%)	5/70 (7.1%)
Hepatocellular carcinoma	2/70 (2.9%) p=0.15	2/69 (3.3%) –	6/69 (8.7%) p=0.13	4/70 (5.7%) –
Females				
Neoplastic nodules	1/70 (1.4%)	1/70 (1.4%)	5/70 (7.1%)	2/70 (2.9%)
Hepatocellular carcinoma	0/70 (0) p=0.0004	0/70 (0) –	5/70 (8.3%) p=0.029	7/70 (10%) p=0.007

[a] Adapted from CPSC 2001.
[b] Statistics for pairwise comparison of treated and control incidences by the Fisher exact test are given beneath incidence values for treated animals. Statistics for exact trend tests are given beneath control incidences.

2. Mechanistic Data

It has been proposed that DINP and other peroxisome proliferators induce liver tumors in rodents by a mechanism or mechanisms directly linked to peroxisome proliferation and related pleiotropic responses. The possible mechanisms by which peroxisome proliferators induce hepatocellular tumors are discussed below (section 3, Mechanism and Human Relevance). This section summarizes data demonstrating that DINP is a peroxisome proliferator and other mechanistic information. Endpoints that may be related to peroxisome proliferation and/or carcinogenic mechanisms include hepatomegaly, peroxisome proliferation, hepatocellular hypertrophy, effects on cell cycle regulation, and gap junction intercellular communication (GJIC). Data relating to these effects are summarized in Table VII-6.

a. Hepatomegaly and Hepatocellular Hypertrophy

Hepatomegaly is an effect of peroxisome proliferation that is due to increases in both cell number and cell size (reviewed in IARC 1995). Hepatomegaly was observed in mice following as little as one week of exposure (Valles et al. 2000) and in rats as early as three weeks (Barber et al. 1987). In chronic studies, hepatomegaly was observed following exposure to dietary levels of 0.3 percent in Fischer 344 rats (Lington et al. 1997; Moore 1998a) and 0.4 percent in male B6C3F1 mice (Moore 1998b). Hepatomegaly was observed in SV129 wild type, but not PPARα-null mice (Valles et al. 2000).

Hepatocellular enlargement may contribute to the hepatomegaly induced by peroxisome proliferation (IARC 1995). Centrilobular to midzonal or diffuse hepatocellular enlargement were observed as early as six months and at doses as low as 0.6% in chronic studies (Lington et al. 1997; Moore 1998a,b).

Table VII-5. Incidence of hepatocellular neoplasia in a 2-year dietary study of DINP-1 in B6C3F1 mice (Moore, 1998b)[a]

Lesion	Percent DINP in feed					
	0	0.05	0.15	0.4	0.8	0.8 R[b]
Males						
Carcinoma Overall incidence At 79 weeks Poly 3[c,d]	10/70 0/15 p<0.001	8/67 0/14 –	10/66 1/13 –	17/65 2/14 p=0.067	20/70 3/15 p=0.017	12/50[e] (p=0.13)
Carcinoma or adenoma Overall incidence At 79 weeks Poly 3	16/70 1/15 p<0.001	13/67 1/14 –	18/66 4/13 –	28/65 3/14 p=0.008	31/70 4/15 p=0.002	–
Females						
Carcinoma Overall incidence At 79 weeks Poly 3	1/70 0/15 p<0.001	2/68 1/15 –	5/68 0/14 p=0.107	7/67 0/14 p=0.024	19/70 2/15 p<0.001	8/50[e] (3.8×10^{-5})
Carcinoma or adenoma overall Overall incidence At 79 weeks Poly 3	3/70 0/15 p<0.001	5/68 1/15 –	10/68 1/14 p=0.043	11/67 0/14 p=0.014	33/70 3/15 p<0.001	–

[a] Adapted from CPSC 2001.
[b] Recovery group. Animals were exposed for 78 weeks, followed by a 26-week recovery period.
[c] Significance value for trend is given in the column for the control group.
[d] Statistical analysis was provide by the National Toxicology Program. Additional statistical tests are found in CPSC 2001, Appendix B, part B.
[e] Level of significance for the recovery group computed by Fisher Exact test. (Statistical tests were not run by NTP on the recovery group.)

b. Peroxisome Proliferation

Peroxisome proliferation is generally assayed by monitoring the induction of peroxisomal beta-oxidation, for example, by measuring palmitoyl-CoA oxidase activity. In some cases, peroxisome number and volume density were ascertained by electron microscopy. Peroxisome proliferation has been observed in rats and mice following as little as one or two weeks of dietary exposure (Moore 1998a; Smith et al. 2000; Valles et al. 2000).

Peroxisome proliferation was evaluated during the course of chronic studies in rats. In the Covance study, peroxisome proliferation was evaluated in the controls and high dose groups by palmitoyl-CoA oxidase at 1, 2, 13, and 104 weeks, and in the mid-high dose

group at 104 weeks (Moore 1998a). Levels of this enzyme were elevated at all time points in both sexes at the high dose (1.2%), and in females at 104 weeks at a dose of 0.6%. The relative increase in enzyme activity at terminal sacrifice was about 4-fold in males and 2.5-fold in females at 1.2% DINP. Lington et al. reported that there was no treatment-related peroxisome proliferation (by electron microscopy) at doses up to 0.6% DINP (Lington et al. 1997). Thus, in these studies, peroxisome proliferation was not observed at doses below 0.6% and inconsistently at 0.6% DINP. Peroxisome proliferation generally correlated with tumorigenesis. Clear increases in hepatocellular tumors were observed only at 1.2%. However, in the Biodynamics study with DINP-A, a small, but statistically significant increase in carcinomas was found in females at 0.5% (CPSC 2001).

In studies of shorter duration, significant increases in peroxisome proliferation were reported at doses as low as 56 mg/kg-d (roughly equivalent to 0.1%) in rats (Jansen et al. 1992). In a 21-day study, a linear dose response was apparent with a range of dialkyl phthalates, although statistical analyses to derive NOEL's were not reported (Barber et al. 1987; Lin 1987).

Induction of peroxisomal beta-oxidation activity has been demonstrated in rat hepatocytes *in vitro* (Benford et al. 1986; Hasmall et al. 1999). In one study, monoisononyl phthalate (MINP), which is believed to be the proximate peroxisome proliferator, was more potent than DINP (Benford et al. 1986; compare also Mitchell et al. 1985).

In the Covance study in mice, peroxisome proliferation was evaluated by palmitoyl-CoA oxidase (control and high dose groups only) activity at 78 and 104 weeks (Moore 1998b). Palmitoyl-CoA oxidase activity was elevated at both time points and in both sexes at the high dose (0.8% DINP). In this study, statistically significant increases in hepatocellular tumors were reported in males at 0.4% DINP and in females at 0.15%. At 104 weeks, palmitoyl-CoA oxidase levels were increased by about 8-fold at the high dose, relative to the controls.

In studies of shorter duration, peroxisome proliferation was reported to occur in mice at doses as low as 336 mg/kg-d (roughly equivalent to 0.5%) DINP (Wolfe et al. 1992). In a 4-week study in B6C3F1 mice at the same doses as the Covance study, the lowest dose at which peroxisome proliferation (by palmitoyl-CoA oxidase and microscopy) was significantly increased was 0.15 percent DINP (Bahnemann 2000; Kaufmann et al. 2001). Thus, the NOEL was 0.05 percent DINP. Although indicators of peroxisome proliferation were not significantly elevated above background at the low dose in this and other studies (e.g. Smith et al. 2000), the data are consistent with a linear dose response.

In rats and mice, peroxisome proliferation is a sensitive, rapidly induced endpoint. Dose response data are consistent with a linear dose response at high dose levels (Bahnemann 2000; Barber et al. 1987; Kaufmann et al. 2001; Lin 1987). Statistically significant increases in the incidence of hepatocelluar tumors have been reported only at doses at which peroxisome proliferation is also observed. This tends to support the hypothesis

that hepatocellular tumors induced by DINP and other peroxisome proliferators are due to peroxisome proliferation.

The apparently linear dose response for peroxisome proliferation may have implications for the tumor dose response, at least in mice and rats, if the tumor incidence is directly related to peroxisome induction. Figure VII-1 shows the risk of hepatocellular tumors in female mice, which are the most sensitive species/sex. Although the incidence of adenoma or carcinoma was significantly increased over background at only three of four doses, and the incidence of carcinoma was significantly elevated at only two doses, the dose response is consistent with a linear (one-hit model) relationship at high doses. Although the shape of the cancer dose response curve at low doses should not be inferred from the dose response at high doses, a linear dose response cannot be ruled out. Alternatively, there could be a minimum relative induction of peroxisome proliferation that is required for tumorigenesis (Budroe et al. 1992; Lake 1995). The dose responses of other events associated with peroxisome proliferation, such as cell proliferation and inhibition of apoptosis, may also influence the cancer dose response (Budroe et a. 1992; Wada et al. 1992).

Limited data on DINP-induced peroxisome proliferation are available in non-rodent species. Hall et al. (1999) reported that there was no increase in palmitoyl-CoA oxidase activity when marmosets were given 2500 mg/kg-d DINP by gavage for 13 weeks. Benford et al. (1986) reported a roughly 3-fold increase in palmitoyl-CoA oxidase and laurate hydroxlylation activity in marmoset hepatocytes treated with up to 0.5 mM MINP. The authors did not report statistical analyses to determine whether the increase was statistically significant. However, the same enzyme activities were induced roughly 7-fold in rat hepatocytes at the same doses.

DINP-induced peroxisome proliferation was also investigated in cynomolgus monkeys (Pugh et al. 2000). Peroxisomal beta-oxidation was increased by 1.5-fold following exposure to 500 mg/kg-d DINP for 14 days, but the increase was not statistically significant. For comparison, statistically significant increases in peroxisomal beta-oxidation were reported at doses as low as 56 mg/kg-d in rats (Jansen et al. 1992) and 336 mg/kg-d in mice (Wolfe et al. 1992).

c. Effects on Cell Cycle Regulation

Peroxisome proliferators may also act by stimulating hepatocellular proliferation and/or by inhibiting apoptosis (Cattley et al. 1998; CPSC 2001; IARC 1995). In the Covance rat study, hepatocellular proliferation was increased at the high dose following 1 week of exposure, but not at 2, 13, or 104 weeks (Moore 1998a). This suggests that DINP induces the acute phase of hepatocellular proliferation, but not the chronic phase. Acute phase proliferation is sufficient to contribute to hepatomegaly, but its role in tumorigenesis is uncertain. In a subchronic study, DINP-1 at 1.2 percent in the diet induced hepatocellular proliferation at 2 weeks, but not at 4 weeks (Smith et al. 2000). In the same study, DINP-A was active at both 2 weeks and 4 weeks.

In the Covance mouse study, DINP did not induce hepatocellular proliferation at the high dose at 78 or 104 weeks; earlier time points were not tested (Moore 1998b). In a subchronic study in mice, DINP-1 at 0.6 percent in the diet induced hepatocellular proliferation at 2 weeks, but not at 4 weeks (Smith et al. 2000). In the same study, DINP-A was not active at either 2 weeks or 4 weeks.

Hasmall et al. (1999) studied the ability of DINP to stimulate DNA synthesis and inhibit apoptosis in rat and human hepatocytes. DINP stimulated DNA synthesis and inhibited apoptosis in rat hepatocytes exposed to 250 to 750 μM DINP for 48 hours. In human hepatocytes from one of three subjects, there was a small, but statistically significant increase in mitotic index at 500 μM DINP, but not at 150 or 750 μM. DINP also failed to suppress apoptosis in human hepatocytes.

d. Gap Junction Intercellular Communication

The mechanistic role of gap junction intercellular communication (GJIC) in nongenotoxic carcinogenesis is uncertain, but it may involve the regulation of cell proliferation. Numerous nongenotoxic carcinogens have been shown to inhibit GJIC (Trosko et al. 1990; Yamasaki 1990). DINP-1 and DINP-A inhibited GJIC in rats following 2 weeks of exposure and in mice following 4 weeks of exposure (Smith et al. 2000). However, DINP was not active in cynomolgus monkeys following 2 weeks of exposure at 500 mg/kg-d (Pugh et al. 2000).

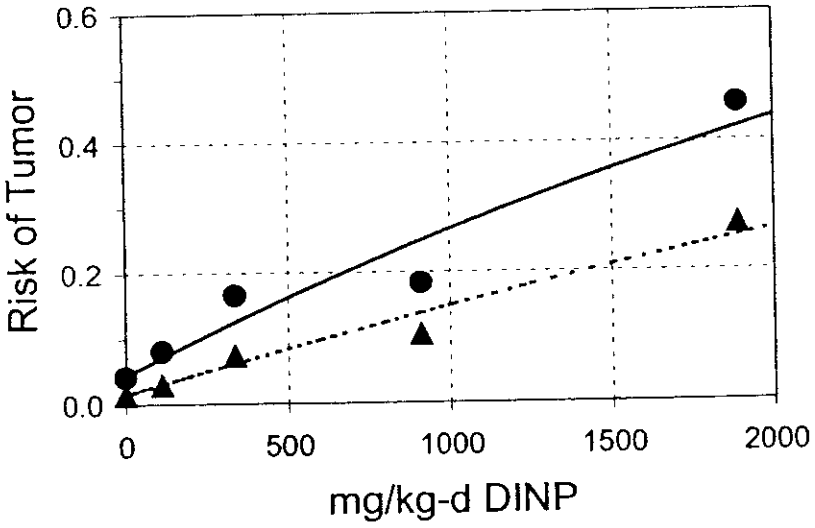

Figure VII-1. Risk of tumor in female B6C3F1 mice exposed to DINP for two years (Moore 1998b): circles, carcinoma or adenoma, observed; solid line, carcinoma or adenoma, one-hit model; triangles, carcinoma, observed; broken line, carcinoma, one-hit model.

Table VII-6. Mechanistic data relating to DINP-induced hepatocellular tumorigenesis

Endpoint	Species, strain	Duration	NOEL [a, b]	LOEL	Reference
Hepatomegaly	Rat, F344	21 days	ND [c]	ND	Barber et al. 1987
		4 weeks	0.1%	1.2%	Smith et al. 2000
		13 weeks	0.1%	0.3%	Bird et al. 1986
		13 weeks	0.25%	0.5%	Myers 1991
		2 years	0.03%	0.3%	Lington et al. 1997; Moore 1998a
	Rat, SD	2 years	0.05%	0.5%	Biodynamics 1986
	Mouse, SV129	1 week	ND	0.8%	Valles et al. 2000
	Mouse, B6C3F1	2 weeks	0.05%	0.6%	Smith et al. 2000
		13 weeks	0.4%	1.0%	Moore 2000
		2 years	0.15%	0.4%	Moore 1998b (males only)
	Cynomolgus monkey	14 days	500 mg/kg-d [d]	ND	Pugh et al. 2000
	Marmoset	13 weeks	2500 mg/kg-d [d]	ND	Hall et al. 1999
Hepatocellular enlargement	Rat, F344	6 to 24 months	ND	0.6%	Lington et al. 1997
		24 months	0.6%	1.2%	Moore 1998a
	Mouse, B6C3F1	24 months	0.4%	0.8%	Moore 1998b
Peroxisome proliferation	Rat, F344	2, 4 weeks	0.1% [e]	1.2%	Smith et al. 2000 (DINP-1, DINP-A)
		21 days	ND [c, e, f]	ND	Barber et al. 1987; Lin 1987
		24 months	0.6% [f]	ND	Lington et al. 1997
		1, 2, 13 weeks	ND	1.2% [e]	Moore 1998a
		104 weeks	ND	0.6%	Moore 1998a (females > males)
	Rat *in vitro*	48 hours	ND [e]	250 µM	Hasmall et al. 1999
		96 hours	ND [e, g]	ND	Benford et al. 1986 (MINP>DINP)
	Mouse, SV129	1 week	ND	0.8% [h]	Valles et al. 2000
	Mouse, B6C3F1	2, 4 weeks	0.05% [e]	0.6%	Smith et al. 2000 (DINP-1 and DINP-A)
		4 weeks	0.05% [e, f]	0.15%	Bahnemann 2000; Kaufmann et al. 2001
		78, 104 weeks	ND	0.8% [e]	Moore 1998b
	Cynomolgus monkey	14 days	500 mg/kg-d [d, e]	ND	Pugh et al. 2000
	Marmoset	13 weeks	2,500 mg/kg-d [d, e]	ND	Hall et al. 1999
	Marmoset *in vitro*	96 hours	ND [e, g]	ND	Benford et al. 1986 (MINP>DINP)
	Human *in vitro*	48 hours	750 µM	ND	Hasmall et al. 1999

Table VII-6. Mechanistic data relating to hepatocellular tumor induction by DINP (continued)

Endpoint	Species/ strain	Duration	NOEL [a, b]	LOEL	Reference
Hepatocellular proliferation	Rat, F344	1 week	0.6%	1.2%	Moore 1998a
		2 weeks	0.1%	1.2%	Smith et al. 2000 (DINP-1, DINP-A)
		4 weeks	1.2%	ND	Smith et al. 2000 (DINP-1)
		4 weeks	0.1%	1.2%	Smith et al. 2000 (DINP-A)
		2, 13, 104 weeks	1.2%	ND	Moore 1998a
	Rat *in vitro*	48 hours	ND	250 µM	Hasmall et al. 1999
	Mouse, B6C3F1	2 weeks	0.05%	0.6%	Smith et al. 2000 (DINP-1)
		2 weeks	0.6%	ND	Smith et al. 2000 (DINP-A)
		4 weeks	0.6%	ND	Smith et al. 2000 (DINP-1, DINP-A)
		78, 104 weeks	0.8%	ND	Moore 1998b
	Human *in vitro*	48 hours	750 µM	ND	Hasmall et al. 1999
Decreased apoptosis	Rat *in vitro*	48 hours	ND	250 µM	Hasmall et al. 1999
	Human *in vitro*	48 hours	750 µM	ND	Hasmall et al. 1999
GJIC	Rat, F344	2 weeks	0.1%	1.2%	Smith et al. 2000 (DINP-1, DINP-A)
		4 weeks	1.2%	ND	Smith et al. 2000 (DINP-1)
		4 weeks	0.1%	1.2%	Smith et al. 2000 (DINP-A)
	Mouse, B6C3F1	2 weeks	0.6%	ND	Smith et al. 2000 (DINP-1)
		2 weeks	0.05%	0.6%	Smith et al. 2000 (DINP-A)
		4 weeks	0.05%	0.6%	Smith et al. 2000 (DINP-1, DINP-A)
	Cynomolgus monkey	14 days	500 mg/kg-d [d]	ND	Pugh et al. 2000
Tumorigenesis	Rat, F344	2 years	0.6%	ND	Lington et al. 1997
		2 years	0.6%	1.2%	Moore 1998a
	Rat, SD	2 years	0.05%	0.5%	Bio/dynamics 1986 (DINP-A) (females)
	Mouse, B6C3F1	2 years	0.05%	0.15%	Moore 1998b (females more sensitive)

Table VII-6. Mechanistic data relating to hepatocellular tumor induction by DINP (continued)

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[a] GJIC, gap junctional intercellular communication; LOEL, lowest observed adverse effect level; ND, not determined; NOEL, no observed adverse effect level.

[b] Doses levels are percent DINP in feed, unless otherwise specified. Where there are differences between sexes, data are for the more sensitive sex.

[c] Animals were given dietary levels of 0.3, 0.6, 1.2, and 2.5% DINP. Data were presented graphically; NOEL's/LOEL's were not reported, but there was a clear increase in liver weight and palmitoyl CoA oxidase with increasing dose.

[d] By gavage.

[e] By peroxisomal beta-oxidation activity.

[f] By electron microscopy.

[g] Cultures were treated with 0, 0.1, 0.25, or 0.5 mM DINP or MINP. Data were presented graphically; NOEL's/LOEL's were not reported. MINP was a more potent palmitoyl CoA oxidase inducer than DINP in rat hepatocytes. Both compounds were weak inducers in marmoset hepatocytes.

[h] By Western blot analysis of acyl-CoA oxidase and Cyp4a induction.

3. Mechanism and Human Relevance

It has been proposed that DINP and many other compounds induce liver tumors in rodents by a mechanism or mechanisms associated with the process of peroxisome proliferation (reviewed in Ashby et al. 1994; Cattley et al. 1998; IARC 1995). While there has been considerable debate regarding the mechanism(s) by which peroxisome proliferation may induce liver tumors (Rusyn et al. 2000), a substantial amount of data has now accumulated that is consistent with the role of peroxisome proliferation in hepatocarcinogenesis in rats and mice. At the same time, there are few or no data demonstrating that peroxisome proliferation may be induced in humans or other primates. This section will briefly discuss possible mechanisms by which peroxisome proliferators may induce cancer in the rodent liver and the relevance of peroxisome proliferation to human risk assessment. These subjects have been reviewed in detail by the CHAP (CPSC 2001) and by other authors (Ashby et al. 1994; Cattley et al. 1998; Corton et al. 2000; Gonzalez et al. 1998; IARC 1995; Lake 1995; Rusyn et al. 2000; Yeldandi et al. 2000). For the most part, this section summarizes information presented in other reviews.

Peroxisome proliferators are characterized by their ability to induce an increase in the size and number of peroxisomes, and associated pleiotropic responses (reviewed in CPSC 2001; IARC 1995). Peroxisomes are single membrane-bound subcellular organelles that contain fatty acid β-oxidation activity, and which are present in virtually all eukaryotic cells (Alberts et al. 1983). Peroxisome proliferation is also accompanied by hepatomegaly; induction of the peroxisomal β-oxidation system, certain cytochrome P_{450} type (microsomal) isozymes (CYP4A), and microsomal and cytosolic epoxide hydrolase; stimulation of protein kinase C; reduction of the activities of glutathione peroxidase, glutathione S-transferase, and superoxide dismutase; and lipofuscin accumulation (IARC 1995). The hepatomegaly (liver enlargement) is due to both hepatocyte hyperplasia (increased cell number) and hypertrophy (increased cell size). The induction of oxidative enzymes may result in increased hydrogen peroxide production and increased metabolism of fatty acids. Protein kinase C is associated with cell proliferation. Glutathione S-transferase and superoxide dismutase help to protect the cell against oxidative damage.

Peroxisome number and volume density remain fairly constant under various physiological and pathological conditions (Yeldandi et al. 2000). In liver parenchymal cells, peroxisomes normally occupy less than two percent of the cytoplasmic volume. Following exposure to peroxisome proliferators, the number and volume of peroxisomes increase in rat and mouse liver to the extent that they may occupy as much as 25% of the hepatocyte cytoplasmic volume.

Peroxisome proliferators are a diverse group of synthetic and naturally occurring compounds, including hypolipidemic drugs (e.g., clofibrate, nafenopin, ciprofibrate, fenofibrate, gemfibrozil, Wy-14,643), leukotriene antagonists, dialkyl phthalates (e.g., DEHP), herbicides, solvents, and the naturally occurring steroid dehydroepiandrosterone (Gonzalez et al. 1998; IARC 1995; Kawashima et al. 1983; Lake 1995; Reddy and Lalwani 1983). The only common structural feature of these compounds appears to be the presence of, or the ability to be metabolized to, a carboxylic acid group or derivative

(Ashby et al. 1994). The pleiotropic responses induced by peroxisome proliferators are qualitatively similar in mice and rats. However, the potency of peroxisome proliferators varies considerably, with the fibrate drugs being among the most potent and phthalate esters being relatively weak (Ashby et al. 1994; IARC 1995). For example, fenofibrate was reported to be 30-fold more potent than DINP (Barber et al. 1987).

Peroxisome proliferators generally exhibit little or no evidence of genotoxicity in standard assays (Galloway et al. 2000). Thus, it has been proposed that the sustained induction of peroxisome proliferation and related responses lead to oxidative stress and the accumulation of indirect mutations (reviewed in Cattley et al., 1998; Conway et al., 1989; CPSC 2001; IARC 1995; Yeldandi et al. 2000). Other responses induced by peroxisome proliferators that may also contribute to carcinogenesis include increased cellular proliferation and a concomitant inhibition of apoptosis. The non-parenchymal (Kupffer) cells of the liver may also play a role in initiating hepatocellular proliferation. These mechanisms are not mutually exclusive, rather, they may act in concert to induce carcinogenesis.

a. Peroxisome Proliferator-Activated Receptor-α

Reddy and Lalwani (1983) were the first to propose the existence of a specific receptor(s) responsible for the action of peroxisome proliferators. A peroxisome proliferator-activated receptor (PPAR) was first identified and cloned in mice (Isseman and Green 1990). Three isoforms of PPAR (α, β/δ, and γ), which is a member of the steroid hormone nuclear receptor superfamily, have been identified, although only PPARα mediates peroxisome proliferation (Gonzalez 1997). It has been proposed that a heterodimeric receptor complex comprised of PPARα and the retinoid X receptor is activated by peroxisome proliferators and 9-*cis*-retinoic acid (Cattley et al. 1998). The activated complex binds to a specific base sequence, or response element, located in the promoters of peroxisome proliferator-responsive genes. The induction of some of the critical enzymes of the peroxisomal, microsomal, and mitochondrial fatty acid oxidation systems by peroxisome proliferators is transcriptionally controlled by PPARα. The PPARα response element (PPRE) is recognized by other nuclear hormone receptors, which may modulate the effects of PPARα on gene expression. Recently, several coactivators or corepressors have been identified that may further modulate the effects of PPARα on gene expression (Reddy 2001; Yeldandi et al. 2000). These cofactors may contribute to the species- and tissue-specific induction of PPARα-responsive genes. Although humans express PPARα at a lower level than mice, the human PPARα was shown to function normally in mouse cells *in vitro* (Cattley et al. 1998).

The mouse PPARα gene has been isolated and a strain of PPARα-null mice, the so-called "knockout" mice incapable of expressing PPARα, has been developed (Lee et al. 1995). The PPARα-null mice were characterized by elevated serum cholesterol levels (Peters et al. 1997a), the presence of lipid-containing vesicles in the liver, increased body fat (Lee et al. 1995), and reduced expression of mitochondrial fatty-acid metabolizing enzymes (Aoyama et al. 1998). The constitutive levels of peroxisomal and microsomal enzymes were similar to those of wild-type mice (Aoyama et al. 1998). However, the pleiotropic

effects associated with PPARα induction, including the development of liver tumors, were not observed in PPARα null mice (Lee et al. 1995; Peters et al. 1997a; Ward et al. 1998). Thus, the immediate pleiotropic responses, as well as the delayed hepatocarcinogenic effects, are believed to be dependent upon PPARα activation (CPSC 2001; Gonzalez et al. 1998; Roberts et al. 2000; Rusyn et al. 2000).

When PPARα null mice were fed 0.1 percent Wy-14,643 for 11 months, no liver tumors were observed, whereas the tumor incidence was 100 percent in homozygous wild-type mice (Peters et al.1997b). It is unknown whether the PPARα-null mice would have developed tumors if the exposure had been continued until two years. In contrast, DEHP induced maternal toxicity, embryolethality, and teratogenicity in both PPARα-null and wild-type mice (Peters et al. 1997c). These studies suggest that PPARα is required for the expression of peroxisome proliferation and tumorigenesis, but not reproductive and developmental toxicity.

b. Carcinogenic Mechanisms in Rodents

Oxidative Stress. Peroxisome proliferation may lead to as great as 20- to 40-fold induction of fatty acyl-CoA oxidase, the first enzyme in the classical β-oxidation pathway, along with more modest increases in other peroxisomal oxidases and cytochrome P450 CYP 4A isoforms (reviewed in CPSC 2001; Yeldandi et al. 2000). The substantial increase in oxidase activity is accompanied by small (2- to 3-fold) increases in peroxisomal catalase and decreased glutathione peroxidase. Furthermore, the dicarboxylic acids formed by CYP4A fatty acid ω-oxidation serve as substrates for fatty acyl-CoA. Thus, disproportionate activation of H_2O_2-generating enzymes and H_2O_2-degrading catalase may lead to a state of increased oxidative stress in liver cells (Reddy 1990; Reddy and Lalwani 1983; Reddy et al. 1980). Undegraded H_2O_2 may react with transition metals, leading to hydroxyl radical formation and oxidative DNA damage.

The general correlation of the magnitude of peroxisome proliferation with hepatocarcinogenicity tends to support this mechanism (Ashby et al. 1994). Furthermore, cell lines overexpressing fatty acyl-CoA oxidase underwent morphologic transformation when they were treated with fatty acyl-CoA substrates and produced tumors in nude mice (Chu et al. 1995; Dadras et al. 1998; Okamoto et al. 1997). However, oxidative DNA damage has not been consistently associated with peroxisome proliferation (Cattley et al. 1998; IARC 1995). Therefore, some investigators have proposed that, while oxidative stress may contribute to carcinogenesis, other mechanisms may be equally as important (Gonzalez et al. 1998; Marsman et al. 1988; Roberts 1996; Rusyn et al. 2000).

Cell Cycle Modulation. During the first several days of exposure in rats or mice, peroxisome proliferators stimulate the proliferation of hepatocytes, resulting in an increase in cell number, in what is termed acute cell proliferation. If exposure continues, a chronic cell stimulation may result, in which increased cell proliferation is balanced by a concomitant increase in apoptosis (programmed cell death) (Marsman et al. 1992; Rusyn et al. 2000). Peroxisome proliferators have been demonstrated to inhibit apoptosis (e.g., Roberts 1996; Roberts et al. 2000). The sustained, low level of cell proliferation is

considered to increase the probability that spontaneous DNA damage would be converted into mutations (IARC 1995). Acute cell proliferation (increase in labeling index) was observed with the hypolipidemic drug Wy-14,643, clofibric acid, and DEHP (Marsman et al. 1988, 1992). Chronic cell proliferation was observed with Wy-14,643 (Marsman et al. 1988, 1992). The chronic cell proliferation induced by Wy-14,643 was not limited to preneoplastic foci (see below) and was accompanied by an increase in apoptosis (Marsman et al. 1988). However, chronic cell proliferation was not observed with clofibric acid or DEHP at doses that were tumorigenic in animals (Marsman et al. 1988, 1992). Only the acute phase of hepatocellular proliferation was observed in the cancer bioassay in rats with DINP (Moore 1998a). Therefore, it is likely that chronic cell proliferation is not a prerequisite for the tumorigenicity of peroxisome proliferators.

Enzymatically or histologically altered hepatic foci are believed to be indicative of preneoplastic cells. Some peroxisome proliferators have been shown to promote the growth of specific subtypes of altered hepatic foci. The hypolipidemic drug Wy-14,643 promoted the growth of ATPase-deficient foci in rats previously exposed to the initiator diethylnitrosamine (Cattley and Popp 1989). The drug nafenopin promoted the growth of liver tumors in rats initiated with aflatoxin B_1 and led to an increase in the number of hepatic foci characterized by weak basophilia and a lack of γ-glutamyltranspeptidase activity (Kraupp-Grasl et al. 1990). DEHP inhibited the formation of γ-glutamyltranspeptidase-positive foci (Carter et al. 1992), but promoted the number of ATPase-deficient foci (reviewed in Bentley et al. 1993). That peroxisome proliferators appear to promote only certain types of altered hepatic foci may explain why several promotion assays gave negative results (Bentley et al. 1993).

Role of Kupffer Cells. Because peroxisome proliferation is increased to a greater extent *in vivo* than *in vitro*, it has been proposed that cytokines from nonparenchymal (Kupffer) cells may be involved in the generation of intercellular signals leading to hepatocyte proliferation (reviewed in CPSC 2001; Rusyn et al. 2000). Kupffer cells (hepatic macrophages) are a source of mitogens, such as TNF-α. Treatment of cell cultures with antibodies to TNF-α (Bojes and Thurman 1996) or Kupffer cell inhibitors (Rose et al., 1997) prevented the stimulation of hepatocyte proliferation by Wy-14,643. However, TNF-α null mice were not refractory to peroxisome proliferator induced cell proliferation, suggesting that other factors may mediate the proliferative response (Lawrence et al., 2001).

Treatment of Kupffer cells with Wy-14,643 led to superoxide production, leading to the induction of the transcription factor NF-κB by a NADPH oxidase-dependent process (reviewed in CPSC 2001; Rusyn et al. 2001). NF-κB is a regulator of TNF-α. In addition, this process is apparently independent of PPARα, because Kupffer cells do not express PPARα and hydroxyl radicals were induced in PPARα knockout mice (Peters et al. 2000). It is likely that cytokines released from Kupffer cells and PPARα are both required for the proliferative response and cancer (Parzefall et al. 2001).

Cancer Mechanism in Mice and Rats. Many details regarding the mechanism by which peroxisome proliferators induce hepatocellular tumors in mice and rats remain to be

elucidated (Corton et al. 2000; CPSC 2001; Roberts et al. 2000; Rusyn et al. 2000, 2001; Yeldandi et al. 2000). However, as discussed by the CHAP, there is a substantial amount of evidence that events downstream of PPARα activation—including oxidative stress, cell proliferation (with or without Kupffer cell involvement), and suppression of apoptosis—lead to liver cancer in rodents (CPSC 2001).

c. Species Differences in Response to Peroxisome Proliferators

While rats and mice are highly responsive to peroxisome proliferators, Syrian hamsters are moderately responsive, and guinea pigs, dogs, marmosets, and rhesus monkeys are generally less responsive or nonresponsive (Cattley et al. 1998; CPSC 2001; IARC 1995; Lake 1995). For example, when Syrian hamsters and rats were exposed to Wy-14,643, peroxisome proliferation was increased in both the rats and hamsters, but hepatocellular proliferation was substantially increased only in rats (Lake et al. 1993). Peroxisome proliferation has been studied in cats, rhesus monkeys, cynomolgus monkeys, marmosets, pigeons, and chickens exposed to peroxisome proliferators. Increased liver weight was accompanied by evidence of peroxisome proliferation in some cases (Reddy et al. 1984; Lalwani et al 1985), but not in others (Tucker and Orton 1993). These observations suggest that the differences between species may be quantitative, rather than qualitative (CPSC 2001).

Liver biopsies from humans taking hypolipidemic drugs generally showed no evidence of peroxisome proliferation (De La Iglesia et al. 1982; Blumcke et al. 1983; Gariot et al. 1987). However, clofibrate was reported to have a statistically significant 50% increase in peroxisome number and a nonsignificant 23% increase in peroxisome density (Hanefield et al. 1983; see also PDR 2000). Furthermore, these studies were limited by the health and nutritional status of controls and treated subjects (CPSC 2001).

A number of studies have demonstrated effects associated with peroxisome proliferation—peroxisomal enzyme induction, stimulation of DNA synthesis, and suppression of apoptosis—in cultured rodent hepatocytes (reviewed in CSPC 2001). Such effects generally have not been observed with human or primate hepatocytes. However, acyl-CoA oxidase activity has been reported to be induced in human hepatocytes by clofibrate or ciprofibrate (Perrone et al. 1998; Scotto et al. 1995). In one study, the induction of acyl-CoA oxidase was accompanied by an increase in apoptosis and decreased DNA synthesis, leading the authors to conclude that human cells would be refractory to carcinogenesis induced by peroxisome proliferators (Perrone et al. 1998). *In vitro* studies with cultured hepatocytes are generally limited by the absence of Kupffer cells, which may play a role in signaling hepatocytes to divide (see above).

PPARα. Endogenous ligands of PPARα include fatty acids and fatty acid derivatives, arachidonic acid-derived prostaglandins, and eicosanoids (reviewed in CPSC 2001). Hypolipidemic drugs are believed to activate PPARα in the liver of humans and rodents. Activation of human PPARα leads to increased levels of apolipoprotein A-II and lipoprotein lipase transcription, and reduced apolipoprotein C-III, which are responsible for their lipid-lowering activity (Auwerx et al. 1996; Staels et al. 1997; Vu-Dac et al.

1995). Fatty acid transport protein and acyl-CoA synthetase are also induced (Martin et al. 1997). However, peroxisome proliferation does not seem to occur. Therefore, it appears that humans have a functional PPARα receptor, but that the cellular responses to receptor activation are different in comparison to rodents.

Transient transfection experiments show that human PPARα can transactivate PPRE reporter constructs, further supporting the conclusion that humans possess a functional PPARα (Sher et al. 1993). PPRE's have been described in human genes that are known to be regulated by PPARα in rodents, including: human apo C-III, lipoprotein lipase, apo A-I, apo A-II, carnitine palmitoyltransferase-I, and acyl CoA oxidase (reviewed in CPSC 2001). Since humans apparently have a functional PPARα and PPRE's, it would be interesting to be able to explain the differences between humans and rodents in their ability to induce peroxisome proliferation.

Several possible mechanisms to explain the lack of peroxisome proliferation in human liver have been suggested. 1) Human liver has less than one-tenth as much PPARα mRNA as mouse liver (Palmer et al. 1998). Thus, it has been proposed that the level of PPARα in the human liver may be sufficient to activate target genes involved in lipid homeostasis, but insufficient to induce peroxisome proliferation. 2) Truncated or mutant PPARα transcripts have also been described, suggesting that mutant, non-functional PPARα variants may also help to lower sensitivity of humans to peroxisome proliferators (reviewed in CPSC 2001). 3) The possibility of polymorphisms in the human PPRE has been suggested (CPSC 2001). 4) Finally, several coactivators or corepressors have been identified that may further modulate the effects of PPARα on gene expression (Reddy 2001; Yeldandi et al. 2000). These cofactors may contribute to the species- and tissue-specific induction of PPARα-responsive genes.

To summarize, it is clear that humans possess a functional PPARα that can be activated by endogenous ligands, hypolipidemic drugs, and probably environmental exposures. However, it is also apparent that some of the genes regulated by PPARα in humans differ from those regulated by PPARα in rodents. Thus, peroxisome proliferation is less easily, or not at all, inducible in humans (CPSC 2001).

Carcinogenicity Studies. A few carcinogenicity studies of peroxisome proliferators in species other than the mouse or rat have been reported (IARC 1995; Cattley et al. 1998). While rats and mice are susceptible to hepatocarcinogenesis following chronic exposure to peroxisome proliferators (Ashby et al. 1994; Bentley et al. 1993; Lake 1995; Reddy and Lalwani 1983), other species appear resistant. For example, the peroxisome proliferators nafenopin and Wy-14,643 induced liver tumors in rats, but not Syrian hamsters following 60 weeks of exposure (Lake et al. 1993). However, it is possible that with a lifetime exposure tumors might have been observed in the hamster. Some hypolipidemic drugs failed to induce liver tumors in non-human primates (Cattley et al. 1998). However, these experiments were limited by the small number of animals and less than half-lifetime of exposure (e.g., Tucker and Orton 1993). A few studies of limited duration in humans taking hypolipidemic drugs failed to show an increased risk of liver cancer (e.g., Law et al. 1994; Huttunen et al. 1994), although these studies had

insufficient power to evaluate the risk of liver cancer (IARC 1995). Furthermore, site concordance across species cannot necessarily be assumed.

d. Relevance to Humans

While humans possess a functional PPARα receptor and PPRE-inducible genes, it is apparent that humans respond to PPARα ligands by inducing a different suite of genes, in comparison to rodents. For example, humans respond to hypolipidemic fibrate drugs by a PPARα-mediated process, yet there is little or no evidence of peroxisome proliferation in humans. Therefore, although data in humans and other primates are limited, the CHAP concluded that peroxisome proliferation is a process that is not easily induced in humans (CPSC 2001). While the possibility that a sufficiently strong peroxisome proliferator or sufficiently high exposure could induce peroxisome proliferation in humans cannot be ruled out, the CHAP further concluded that it is unlikely that DINP could present a cancer hazard to humans under foreseeable conditions of exposure. The CPSC staff agrees that peroxisome proliferation is not readily induced in humans and that exposure to DINP, a relatively weak peroxisome proliferator in comparison to the fibrate drugs, is unlikely to present a cancer hazard to humans.

B. Kidney

A small number (2/65) of renal tubular cell carcinomas were observed in high dose males in the Covance study in rats (Moore 1998a) (Table VII-7). Although the increase over background (0/65) was not statistically significant, the trend test was positive (p=0.022). In addition, the incidence (4/50) in the recovery group (79-week exposure/26-week recovery) was statistically significant.

A small number of renal tubular cell carcinomas were observed only in males exposed to 1.2 percent DINP. Furthermore, there is experimental evidence that these tumors arose by a mechanism involving the accumulation of α2u-globulin (Caldwell et al. 1999). α2u-Globulin is a protein that is specific to the male rat. Renal tubular cell tumors induced by this mechanism are not considered relevant to human risk assessment (Schaeffer 1991). The higher incidence of these tumors in the recovery group animals suggests that DINP, as other peroxisome proliferators, may inhibit the synthesis of α2u-globulin (Alvares et al. 1996).

Table VII-7. Incidence of renal tubular carcinoma in male Fischer 344 rats in a 2-year dietary study of DINP-1 (Moore 1998a)[a]

	Percent DINP in feed					
Incidence	0	0.05	0.15	0.6	1.2	1.2[b]
Overall incidence	0/65	0/55	0/55	0/65	2/65	4/50
At 79 weeks	0/10	NA	NA	0/10	0/10	–
Poly 3[c, d]	p=0.022	–	–	–	p=0.219	p=0.03[e]

[a] Adapted from CPSC 2001.
[b] Recovery group. Animals were exposed for 78 weeks, followed by a 26-week recovery period.
[c] Significance value for trend (excluding recovery group) is given in the column for the control group. The recovery group was not included in the Poly 3 analysis.
[d] Statistical analysis was provided by the National Toxicology Program. Additional statistical tests are found in CPSC 2001, Appendix B, Part A.
[e] Level of statistical significance computed by Fisher Exact test. (Statistical tests were not run by NTP on recovery group.)

C. Mononuclear Cell Leukemia

Significant increases in the incidence of mononuclear cell leukemia (MNCL) were observed in Fischer 344 rats of both sexes exposed to at least 0.3 percent DINP (Lington et al. 1996; Moore 1998a) (Tables VII-8, 9). Although the incidence of MNCL was low at 79 weeks in the Covance study, it was significantly elevated in the recovery groups (Table VII-9). MNCL has a high spontaneous incidence in Fischer rats, ranging from 10 to 72 percent in National Toxicology Program studies (Haseman et al. 1990), and has increased over time (Haseman et al. 1998). Elevated incidence of MNCL is a common finding in chronic studies in Fischer rats. Due to its high background rate, MNCL is often considered to be of uncertain relevance in the evaluation of the cancer hazard in humans. Furthermore, no hematopoetic neoplasms were found in Sprague-Dawley CD rats treated with DINP-A (Bio/dynamics 1986) or in mice treated with DINP-1 (Moore 1998b). Therefore, MNCL will not be used to predict cancer risk in humans.

Table VII-8. Incidence of mononuclear cell leukemia in a 2-year dietary study of DINP-1 in Fischer 344 (Lington et al., 1997) [a]

	Percent DINP in feed			
	0	**0.03**	**0.3**	**0.6**
Males	33/81 p=0.00003	28/80 –	48/80 p=0.011	51/80 p=0.0028
Females	22/81 p=0.00001	20/81 –	30/80 p=0.11	43/80 p=0.0005

[a] Adapted from CPSC 2001.
[b] Statistics for pairwise comparison of treated and control incidences by the Fisher exact test are given beneath incidence values for treated animals. Statistics for trend tests are given beneath control incidences.

Table VII-9. Incidence of mononuclear cell leukemia in a 2-year dietary study of DINP-1 in Fischer 344 rats (Moore 1998a) [a]

Incidence	Percent DINP in feed					
	0	**0.05**	**0.15**	**0.6**	**1.2**	**1.2 R** [b]
Males						
Overall incidence At 79 weeks Life table [c, d]	22/65 1/10 p=0.002	23/55 NA -	21/55 NA -	32/65 0/10 p=0.027	30/65 0/10 p=0.022	31/50 [e] (p=0.0024)
Females						
Overall incidence At 79 weeks Life table	17/65 0/10 p<0.001	16/49 NA -	9/50 NA -	30/65 1/10 p=0.020	29/65 1/10 p=0.021	24/50 [e] (p=0.013)

[a] Adapted from CPSC 2001.
[b] Recovery group. Animals were exposed for 78 weeks, followed by a 26-week recovery period.
[c] Statistical significance computed by life table analysis, since MCL is a relatively lethal disease. Significance value for trend is given in the column for the control group.
[d] Statistical analysis was provided by the National Toxicology Program. Additional statistical tests are found in CPSC 2001, Appendix B, Part A.
[e] Level of significance for the recovery group computed by Fisher Exact test. (Statistical tests were not run by NTP on the recovery group.)

D. Other Sites

Other peroxisome proliferators have been reported to induce tumors in the testes and pancreas, in addition to the liver by a mechanism that appears to be independent of peroxisome proliferation (e.g., Biegel et al. 2001). In the Lington et al. (1997) and Covance (Moore 1998a) studies of DINP-1 with Fischer 344 rats, interstitial cell tumors of the testes were observed at a high incidence both in control and treated males. No conclusions regarding this tumor site may be made, due to the high background incidence.

In the Bio/dynamics (1986) study of DINP-A with Sprague-Dawley rats, the incidence of testicular interstitial cell hyperplasia was significantly elevated at the high dose (1.0%) relative to both concurrent and historical controls (see also CPSC 2001). Interstitial cell tumors were non-significantly elevated at the high dose (7/60) relative to concurrent (2/59) and historical (3.4 to 23.4%) controls. Other sites with non-significant increases in hyperplasia and/or tumor incidence were the pancreas and endometrium.

E. Summary of Carcinogenicity

In chronic dietary studies, DINP treatment was associated with increased incidences of hepatocellular tumors in rats and mice of both sexes, renal tubular cell carcinoma in male rats, and mononuclear cell leukemia in Fischer 344 rats. As discussed above, however, the hepatocellular tumors are believed to arise by a mechanism (peroxisome proliferation and related effects) that is not easily induced in humans (CPSC 2001). The renal tubule tumors are believed to arise by a mechanism (α2u-globulin) that is unique to male rats (Schaeffer 1991). The MNCL is a neoplasm with a high spontaneous rate in Fischer 344 rats, that is considered of questionable relevance to humans (CPSC 2001). Therefore, the CPSC staff regards DINP to be possibly carcinogenic in humans (rather than probably or known to be), based on limited evidence of carcinogenicity in experimental animals, as defined under the FHSA and implementing regulations (CPSC 1992). The finding that DINP is possibly carcinogenic means that carcinogenicity will not be considered in evaluating the potential risks of DINP exposure to humans.

VIII. Dose Response

Liver is the most sensitive organ site for the toxic effects of DINP (see part IV, above), being more sensitive than the kidney and reproductive/developmental effects. Rats are also more sensitive than mice. Thus, liver effects in the rat have been considered to be the critical endpoint for assessing the chronic effects of DINP (Babich 1998; CPSC 1998; CPSC 2001; Wilkinson and Lamb 1999). The NOAEL for non-cancer effects is 15 mg/kg-d, which is based on increased incidence of spongiosis hepatis and increased serum enzyme levels in male rats (Lington et al. 1997).

The acceptable daily intake (ADI) is an estimate of the amount of chemical a person can be exposed to on a daily basis over an extended period of time (up to lifetime) with a negligible risk of suffering deleterious effects. An uncertainty factor approach is used to derive ADI values for non-cancer endpoints (CPSC 1992). The default procedure is to divide the NOAEL by a net uncertainty factor of 100. This 100-fold factor is the product of a 10-fold factor for interspecies differences and another 10-fold factor for interindividual differences. If a NOAEL has not been established, then the LOAEL is divided by an uncertainty factor of 1,000. In this case, the net uncertainty factor includes an additional 10-fold factor. Previously, the CPSC staff applied a 100-fold uncertainty factor to the NOAEL (15 mg/kg-d) in the Lington study, resulting in an acceptable daily intake (ADI) value of 150 µg/kg-d (CPSC 1998).

The benchmark dose (BMD) may used as an alternative to the NOAEL in setting ADI values (Crump, 1984). The principle advantage of using the BMD is that it is less sensitive to the selection of experimental doses and the number of animals per dose group. Thus, the CHAP derived a benchmark dose (D_{05}) estimate of 12 mg/kg-d by fitting the Lington data to a polynomial model (CPSC 2001; see also Babich and Greene 2000; France 2001). In this case, the benchmark dose is the maximum likelihood estimate (MLE) of the dose at which the extra risk of spongiosis hepatis is 5 percent. The polynomial model is described by (Crump 1984; EPA 2000):

$$P = C + (1 - C) \cdot (1 - e^{-\sum_{i=1}^{n} B_i D^i}) \qquad \text{(VIII.1)}$$

where: P is the probability of lesion; D_i is the dose for the *i*-th group; B_i and C are parameters to be estimated; and $B_i \geq 0$.

The CHAP applied a net uncertainty factor of 100 to the BMD estimate (12 mg/kg-d) from the Lington study to give an ADI of 120 µg/kg-d (CPSC 2001). This is somewhat lower than the ADI derived by the CPSC staff (CPSC1998). Both ADI values are based on liver effects in male rats in the study by Lington et al. (1997). Because both values were derived using accepted methods and neither estimate is clearly superior to the other, the lower value of 120 µg/kg-d is preferred for risk assessment.

No data on the relative susceptibility of children or immature animals to DINP are available. In general, children are more susceptible to some chemicals and less

susceptible to others (Guzelian et al. 1992; NRC 1993). The National Research Council recommended the use of an additional 10-fold uncertainty factor as a default assumption to account for the possible increased susceptibility of children (NRC 1993). In some cases, the use of an additional safety factor is mandated under the Food Protection Act. In 1991, CPSC proposed the use of an additional 10-fold uncertainty factor for children, to be used as a default assumption in the absence of information to the contrary. The proposal was part of the Commission's proposed guidelines for assessing chronic toxicity. However, public comments, including comments from other regulatory agencies, argued that the default 10-fold uncertainty factor for sensitive populations was sufficient to protect children and that the overall risk assessment process was conservative. Therefore, the additional uncertainty factor for children was dropped from the final chronic hazard guidelines (CPSC 1992). The CHAP did not apply an additional uncertainty factor for children (CPSC 2001), and an additional uncertainty factor will not be used in the present risk assessment. However, as noted by the CHAP, the lack of data on the effects of DINP in children or immature animals is a potentially significant source of uncertainty.

IX. Exposure

A. Oral Exposure from Children's Products

DINP and other dialkyl phthalates have been used in children's products such as soft plastic toys and teethers (Chen 1998a, 2002; Health Canada 1998; Marin et al. 1998; Rastogi 1998; Rastogi et al. 1997; Simoneau et al. 2001; Sugita et al. 2001; Vikelsøe et a. 1997). In 1998, DINP was present in 31 of 35 (89%) children's products tested by CPSC (Chen 1998a). Diisooctyl phthalate (Chen 1998a) and DINP (Sugita et al. 2001) have also been used in some pacifiers, although most pacifiers are either latex or silicone. Because plasticizers are not covalently bound to PVC, they may be released when children place PVC products in their mouths. Dialkyl phthalates are not currently used in teethers, rattles, or pacifiers in the U.S.

1. Migration

a. Laboratory Studies

Previous Studies. Several laboratory methods have been used to measure DINP migration from children's products made from PVC. Laboratory methods generally involve extraction with saline or artificial saliva combined with mechanical action, including shaking (Axford et al. 1999; Earls et al. 1998; Fiala et al. 2000; Rastogi et al. 1997; Steiner et al. 1998; Vikelsøe et al. 1997), ultrasound (Fiala et al. 2000; Steiner et al. 1998), impaction (Chen 1998a,b; Health Canada 1998), or tumbling (RIVM 1998; Rijk and Ehlert 1999; Rijk et al. 1999; Simoneau et al. 2001). The variety of methods has led to a broad range of results (Table IX-1). The lack of a standard method and consistent units, as well as few attempts at testing similar products, initially hampered comparisons among methods (Babich 1998).

For example, the Danish National Environmental Institute (NERI) reported phthalate migration rates from teethers as great as 23,000 $\mu g/dm^2/h$* (2,600 $\mu g/11\ cm^2/h$),† using a shaking method (Rastogi et al., 1997). However, using a disk taken from the same teether and the NERI method, the CPSC staff measured migration rates ranging from 7.2 to 102 $\mu g/dm^2/h$ (0.8 to 11 $\mu g/11\ cm^2/h$) (Chen 1998b).

The CPSC staff used an impaction method that was developed to measure DEHP migration from children's products (CPSC 1983). This method uses a reciprocating piston to approximate the effects of a child's biting or chewing. Using the impaction method, CPSC obtained DINP migration rates ranging from 1.0 to 48 $\mu g/11\ cm^2/h$ (Chen 1998a). When CPSC tested the same teether tested by NERI (Rastogi et al. 1997), a migration rate of about 13 $\mu g/11\ cm^2/h$ was obtained by the impaction method, compared to a range of values by the NERI shaking method (0.8 to 11 $\mu g/11\ cm^2/h$) (Chen 1998b).

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* Units: $\mu g/dm^2/h$, micrograms per square decimeter per hour; $\mu g/11\ cm^2/h$, micrograms per 11 square centimeters per hour; $\mu g/10\ cm^2/min$, micrograms per 10 square centimeters per minute.

† Risk assessments typically assume a surface area of 10 to 11 cm^2 (CPSC 1998; RIVM 1998).

A tumbling method developed at the TNO Nutrition and Food Research Institute and subsequently modified by the European Commission Joint Research Center (JRC) has emerged as a candidate standard method. Migration rates obtained by the TNO and JRC methods, often referred to as the "head-over-heels" method, ranged from 0.9 to 5.6 μg/10 cm^2/min (59 to 370 μg/11 cm^2/h) (RIVM 1998; Rijk and Ehlert 1999; Simoneau et al. 2001). The TNO (Rijk and Ehlert 1999; Rijk et al. 1999) and JRC (Simoneau et al. 2001) methods have been evaluated in interlaboratory studies and compare favorably with *in vivo* studies (see below).

Updated CPSC Exposure and Risk Assessment. As part of the process of refining the 1998 risk assessment, the CPSC staff purchased 41 plastic children's products that could be mouthed by small children, including teethers and toys (Chen 2002). Products were selected from the list of objects mouthed in the CPSC observational study (Greene 2002a). Of the 41 plastic children's products, 35 were made of soft plastic, 24 were made either entirely or partly of PVC, and 16 contained DINP. Other plasticizers present in PVC articles were acetyl tributyl citrate (ATBC), di(2-ethylhexyl) adipate (DEHA), and DEHP. Some of these products contained several toys, and some dolls contained parts made of different plastics. Thus, a total of 133 articles were available for testing. Eighty-five of the 133 articles were soft plastic, of which 51 were made of PVC; the remainder contained polyethylene, polypropylene, polystyrene, poly(styrene-acrylonitrile-butadiene), poly(styrene-butadiene), or silicone. Among the PVC articles, 36 of 51 contained DINP. Therefore, 36 of 85 (42%) soft plastic children's articles contained DINP. No teethers contained DINP. Several DINP-containing products were toy food items (Table IX-2).

Of the 36 DINP-containing articles, some were not amenable to testing due to their size or shape. Thus, 24 DINP articles were tested for DINP content and migration rate. The migration rate was measured by the JRC method (Simoneau et al. (2001) (Table IX-2). The DINP content ranged from 12.9 to 39.4 percent by weight, with a mean of 30 percent. Migration rates ranged from 1.0 to 11.1 μg/10 cm^2/min, with a mean of 4.1 ± 2.7 μg/10 cm^2/min (269 μg/11 cm^2/h) and a median of 3.4 μg/10 cm^2/min (Figure IX-1). As observed previously (CPSC 1998), the migration rate did not correlate well with the DINP content (Figure IX-2).

b. Studies with Human Subjects

As noted by the CHAP on DEHP, the CPSC impaction method was not validated (CPSC 1985). This assessment applies equally to many other methods used to measure dialkyl phthalate migration. Thus, some laboratories have attempted to validate their laboratory methods by comparison with human volunteer studies. In the Dutch Consensus Group study, adult volunteers were asked to mouth or gently chew PVC disks or teethers for a defined period of time, during which all saliva was collected (RIVM 1998). Test articles included specially prepared 10 cm^2 disks, a teether, and a 10 cm^2 disk cut from the same type of teether. The average migration rate was 1.8 μg/10 cm^2/min (range 1.38 to 2.4) or about 120 μg/11 cm^2/h (Table IX-3). The mean *in vivo* migration rate with the standard disk was 1.38 ± 0.99 μg/10 cm^2/min. The *in vivo* migration rates were within 2-fold of

migration rates obtained by the TNO method with the same test articles (2.5 to 4.2 μg/10 cm^2/min) (compare Table IX-1).

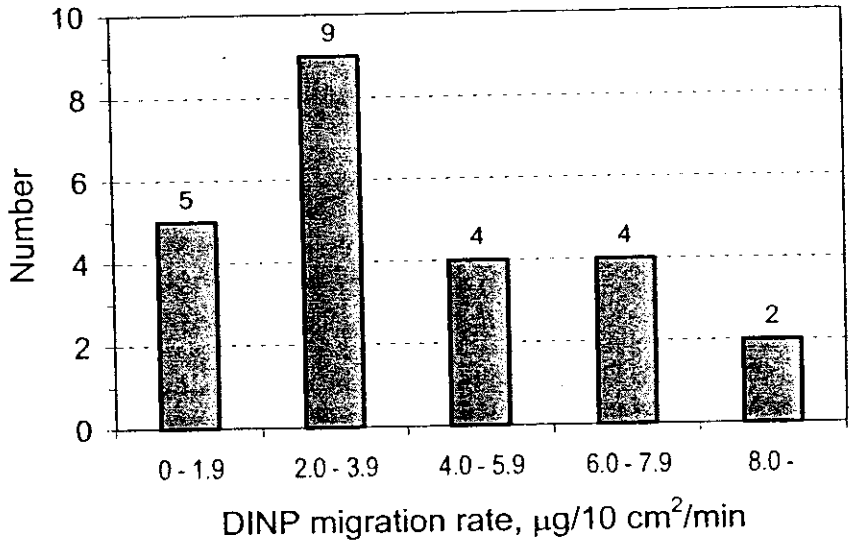

Figure IX-1. Distribution of DINP migration rates (micrograms per 10 square centimeters per minute) as determined by the TNO method (Chen 2002).

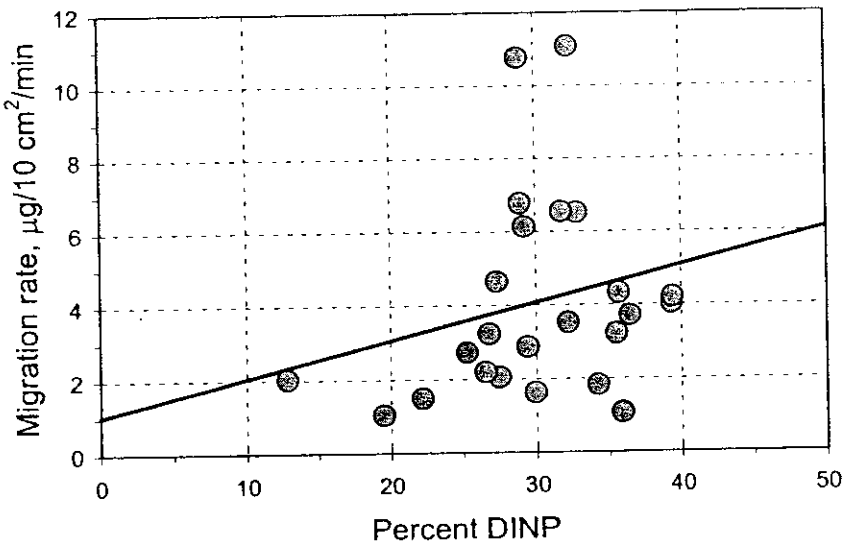

Figure IX-2. Effect of DINP content (percent DINP by weight) on migration rate (micrograms per 10 square centimeters per minute) (Chen 2002).

A standard PVC disk of the same formulation used in the human subject study, but from a new batch, was tested in two interlaboratory studies. In the first study, using the TNO method, the mean migration rate for the standard disk was 2.07 μg/10 cm^2/min (Rijk and Ehlert 1999; Rijk et al. 1999), which is slightly greater than the *in vivo* migration rate of 1.38 μg/10 cm^2/min (RIVM 1998). In a subsequent study, using the JRC method, the amount of saliva simulant used to study DINP migration was increased. In this study, the mean migration rate for the standard disk was 3.66 μg/10 cm^2/min (Simoneau et al. 2001). Thus, it appears that the TNO and JRC methods over predict the *in vivo* migration rate (1.38 μg/10 cm^2/min) by about 1.5- to 3-fold.

The Austrian Standards Institute (ASI) also conducted a human subject study (Fiala et al. 2000; Steiner et al. 1998). In this study, chewing resulted in a roughly 50 percent greater migration rate than sucking. In the Dutch and CPSC studies, volunteers were asked to mouth or gently chew the test article. *In vivo* migration rates were 2- to 3.5-fold greater than the *in vitro* rate obtained by ultrasound and 23- to 37-fold greater than by shaking (compare Table IX-1).

Upon learning of the relatively high *in vivo* migration rates in the Dutch Consensus Group (e.g., 120 μg/11 cm^2/h) and Austrian Standards Institute (e.g., 146 μg/11 cm^2/h) studies, the CPSC staff also conducted tests with human subjects. Ten volunteers tested disks cut from five identical toys. The average migration rate in these tests was 268.1 μg/11 cm^2/h, which was roughly 40-fold greater than the average rate obtained by impaction (6.4 μg/11 cm^2/h) with disks cut from the same five toys (Chen 1998a).* Thus, the CPSC impaction method substantially underestimated the *in vivo* migration rate. To estimate exposure, CPSC applied a correction factor to migration rates measured by the impaction method, but concluded that a new laboratory method was needed (CPSC 1998).

Recently, CPSC used the JRC modification of the TNO method to test disks cut from toys identical to those used in the CPSC human subjects study. The mean migration rate was 7.5 ±0.95 μg/10 cm^2/min (496 μg/11 cm^2/h), which is roughly double the *in vivo* migration rate (241 μg/11 cm^2/h) and 70-fold greater than the rate measured by impaction (6.1 μg/11 cm^2/h) (Chen 2002). Thus, the JRC method yields higher migration rates that more closely approximate the *in vivo* results.

Overall, the human subject studies reported by three laboratories gave roughly comparable results, with overall *in vivo* migration rates ranging from 92 to 205 μg/11 cm^2/h. All three studies were also characterized by considerable inter-individual variation, with almost a 10-fold range in individual migration rates (Table IX-3).

The use of adults as surrogates for children is a potential source of uncertainty in estimating exposure (RIVM 1998). It is possible that adults apply more force than children, which would tend to overestimate exposure (CPSC 1998; RIVM 1998).

* In the 1998 risk assessment, CPSC calculated the mean and ratio from 5 pairs of *in vivo* and *in vitro* migration rates, because the disks tested were obtained from 5 identical toys (CPSC 1998; Greene 1998).

Furthermore, in the human subject studies, all saliva was carefully collected to represent what might be ingested, whereas infants would not necessarily ingest all saliva.

It is also possible that DINP was absorbed through the oral mucosa during the human subject studies (RIVM 1998). This would lead to an underestimate of the *in vivo* migration rate. In the Dutch Consensus Group human subject study with the standard disk, there was an average of about 1 μg/mL DINP in saliva (mean=0.94 ±0.59 μg/mL) and an average of about 15 mL saliva collected during each 15-minute collection period. Thus, there was roughly 15 μg of DINP in each volunteer's mouth at any given time. In rats, 3.4 percent of the applied dose of DINP was absorbed over a 7-day period (Stoltz and El-hawari 1983; Stoltz et al. 1985). The dermal absorption rate was about 0.02 percent per hour. The amount of DINP absorbed during the course of the human subjects study (1 hour) can be estimated by:

$$(15\ \mu g\ DINP)\times\left(2.0\times10^{-4}\ h^{-1}\right)=3.0\times10^{-3}\ \mu g/h\ DINP \qquad \text{(IX.1)}$$

Therefore, the amount of DINP absorbed through the oral mucosa is estimated to be about $3.0\text{x}10^{-3}$ μg/h. The DINP migration rate was 1.38 μg/min or 82.8 μg/h. Thus, the amount of DINP absorbed through the oral mucosa was about 0.004 percent of the DINP migration rate.

It is possible that the oral mucosa may be more permeable than the rat skin, due to hydration or regional variation in permeability (Galey et al. 1976; Wester and Maibach 1983). Galey et al. compared *in vitro* percutaneous absorption among full thickness human skin, human dermis, and canine buccal mucosa. The relative increase in absorption comparing human skin to canine buccal mucosa was greatest with hydrophilic compounds. With estradiol, which is relatively hydrophobic, absorption was about 12-fold greater with canine buccal mucosa than with full thickness human skin. If the oral mucosa were, for example, 10-fold more permeable than rat skin, the amount absorbed would still be small (~0.04%) relative to the migration rate. Therefore, it is likely that the error introduced by absorption through the oral mucosa is small. However, the relative permeability of human oral mucosa to hydrophobic compounds such as DINP is unknown and remains a source of uncertainty.

The CHAP used two different methods to estimate the extent of absorption of DINP through the oral mucosa during children's mouthing of toys and pacifiers (CPSC 2001, Appendix A). These methods are discussed in detail below (section IX.B.). By one method, the CHAP estimated that exposure through the oral mucosa would add from 0.26 μg/kg-d (19 to 36-month-olds) to 3.1 μg/kg-d (0 to 18-month-olds) to the oral ingestion exposure from mouthing soft plastic toys. The CHAP concluded that these additional exposures would be negligible in comparison to the oral exposure by ingestion. By another method, the CHAP estimated that the additional exposure would range from 15 to 150 μg/kg-d for 0 to 18 and 19 to 36-month-olds, respectively. For the reasons discussed below (section IX.B), the CPSC staff concludes that the former method and its resulting exposure estimates are more appropriate in this case.

Table IX-I. Laboratory measurements of DINP migration from PVC children's products [a]

Laboratory	Method	Products	% DINP	N [b]	Units	Mean	(µg/11 cm^2/h)	SD	Range	Reference
Danish National Environmental Research Institute [c, d]	Shaking	Teethers	--	2	µg/g or ppm	--	--	--	89 - 24,691	Vikelsoe et al. 1997
			--	1	µg/dm^2/h	23,260	(2,559)	--	--	Rastogi et al. 1997
TNO	Tumbling (TNO method)	Teether	--	1	µg/10 cm^2/min	3.1	(205)	0.5	2.5 - 4.2	RIVM 1998
LGC [e]	Shaking (1) Shaking (4) Tumbling	Toys	--	2	µg/10 cm^2/min	0.3 1.8 1.6	(20) (119) (106)	0 0.4 1.1	0.3 - 0.3 1.6 - 2.1 0.8 - 2.3	Earls et al. 1998
Health Canada [f]	Impaction	Teethers, toys, pacifiers	3.9 - 44	27	µg/10 cm^2/h	0.32	(0.35)	0.08	--	Health Canada 1998
CPSC [g]	Impaction	Teethers, toys	15.1 - 54.4	31	µg/11 cm^2/h	8.2	(8.2)	9.83	1.0 - 48.1	Chen 1998a
CPSC [h]	Impaction Shaking	Teether	--	1	µg/dm^2/h	119 7.2 - 102	(13) (0.8 – 11)		105 - 133 7.2 - 102	Chen 1998b
TNO [i]	Tumbling (TNO method)	Toys, teethers	21.0 - 46.6	10	µg/10 cm^2/min	2.4	(158)	1.38	0.9 - 5.6	Rijk and Ehlert 1999; Rijk et al. 1999
LGC [j]	Shaking 37°C Shaking 65°C	Teether, toy	--	2	µg/10 cm^2/min	0.95 4.5	(63) (294)	0.35 0.78	0.7 - 1.2 3.9 - 5.0	Axford et al. 1999
Austrian Standards Institute [k]	Static Shaking Ultrasound	Teether	36	1	µg/dm^2/h	12.7 36.3 387.3	(1.4) (4.0) (42.6)	-- -- --	-- -- --	Fiala et al. 2000 Steiner et al. 1998
JRC [l]	Tumbling (JRC method) Shaking, mild Shaking, stringent	Toys, teethers	26 – 41.7	5	µg/10 cm^2/min	4.0 0.89 4.6	(264) (59) (304)	1.45 0.51 2.5	1.9 - 5.4 0.49 - 1.75 2.6 - 8.8	Simoneau et al. 2001
CPSC	Tumbling (JRC method)	Toys	12.9 – 39.4	24	µg/10 cm^2/min	4.1	(269)	2.7	1.0 - 11.1	Chen 2002

Table IX-I. Laboratory measurements of DINP migration from PVC children's products (continued)

[a] Adapted from CPSC 2001.
[b] N, number of articles tested; SD, standard deviation.
[c] Units were in micrograms per square decimeter per day.
[d] CPSC staff were unable to replicate the high value using a disk cut from the same article by either the Danish test method or the CPSC method (Chen 1998b).
[e] Two toys were tested by shaking under various conditions (all at 37°C) or by tumbling (at 20°C). Shaking data for method 1 (no glass beads) and method 4 (glass beads at 200 strokes per minute) are shown. Units were micrograms per 10 square centimeters per minute.
[f] Impaction was with a "bite form" used to test the resistance of toys to breaking. Units were micrograms per 10 square centimeters per hour.
[g] Original units were micrograms per 11 square centimeters per hour.
[h] Teether from Rastogi et al. (1997) tested using the CPSC impaction method and the NERI shaking method.
[i] This is from an interlaboratory study coordinated by the TNO Nutrition and Food Research Institute. Original units were micrograms per minute.
[j] This was from an interlaboratory study coordinated by the Laboratory of the Government Chemist. In this method, glass balls are added to the flask to aid extraction. Units were micrograms per 10 square centimeters per minute.
[k] Original units were micrograms per square decimeter, for either 1 or 3 hours. All values shown here were adjusted to 1 hour.
[l] This is from an interlaboratory study coordinated by the Joint Research Centre. Five toys and a standard disk (not shown) were tested by three methods. Shaking methods were essentially those of Axford et al. (1999). Original units micrograms per square centimeter per minute (Simoneau et al. 2001).

Table IX-2. Migration of DINP from PVC children's products by the JRC method (Chen 2002).

Sample no.	Description	Number tested	% DINP	Migration rate [a] (μg/10 cm^2/min)
17	Doll's face	2	35.5	3.2
67	Corner cushion	3	39.4	4.2
69	Blue seat	6	29.4	2.9
71	Blue doll	3	25.3	2.7
76	Yellow duck	3	29.3	6.1
77	Tub toy	3	35.9	1.1
79	Book	3	22.2	1.5
83	Green whale	3	39.3	4.0
87	Bacon	3	34.2	1.8
87	Donut	3	29.0	6.8
87	Egg	3	36.4	3.7
87	French fries	3	28.9	10.8
87	Ice cream	3	27.5	2.0
87	Lettuce	3	27.4	4.6
87	Pizza	3	32.3	11.1
87	Spaghetti	3	35.7	4.3
87	Tomato	2	31.8	6.6
88	Doll's leg	3	32.8	6.5
91	Doll's face	1	26.6	2.2
92	Cape	3	19.5	1.0
95	Ball (protrusion)	3	26.8	3.2
97	Doll's face	3	32.2	3.5
98	Large reptile	1	12.9	2.0
99	Doll's face	1	30.0	1.6
		Mean	30.0	4.1
		Median	29.7	3.4
		SD	6.2	2.7

[a] Micrograms per 10 square centimeters per minute.

Table IX-3. Human subjects studies of DINP migration from PVC children's products [a]

Laboratory	Method	Products	% DINP	N [b]	Units	Mean	(µg/11 cm^2/h)	SD	Range	Reference
Dutch consensus group [c]	Mouthing	Standard disk, teether	--	20	µg/10 cm^2/min	1.8	(119)	--	1.38 - 2.4	RIVM 1998
Austrian Standards Institute [d]	Sucking	Teether	36	9	µg/dm^2/hour	833	(92)	--	297 - 1,452	Fiala et al. 2000; Steiner et al. 1998
	Chewing		36	9		1330	(146)	--	768 - 5,839	
CPSC [e]	Mouthing	Toy (disk)	43	10	µg/10 cm^2/hour	268.1	(295)	158.1	63 - 597	Chen 1998a

[a] Adapted from CPSC 2001.

[b] N, number of human subjects; SD, standard deviation.

[c] Test articles included disks cut from a specially prepared PVC sheet, a teether, and disks cut from the same type of teether. Units were micrograms per 10 square centimeters per minute.

[d] Original units were micrograms per square decimeter, for either 1 or 3 hours. All values shown here were adjusted to 1 hour.

[e] 20 disks were cut from 5 identical toys. Ten disks were tested by 10 subjects (the remaining 10 were tested by impaction). Units were micrograms per 10.3 square centimeters per hour. In the 1998 CPSC risk assessment, migration rates were treated as 5 pairs of volunteers, because the disks were taken from 5 toys (CPSC 1998; Greene 1998).

2. Mouthing Duration

a. Previous Studies

In the past, risk assessors were forced to make assumptions regarding the amount of time that children mouth objects such as teethers, toys, and pacifiers. For example, CPSC previously assumed that teethers would be used for 2 to 6 hours per day and pacifiers from 4 to 12 hours (CPSC, 1983). Recently, however, several observational studies of children's mouthing activity have been reported (Table IX-4). Zartarian et al. (1998) used a video camera to record the mouthing activities of two 2-year-old and two 4-year-old children for one day. They reported daily mouthing times for non-food objects of 7 and 28 minutes for the 2-year-olds and 16 and 22 minutes for the 4-year-olds. The objects mouthed included hands, toys, and hard surfaces.

Table IX-4. Observational studies of children's mouthing activity

Study	Age range	N [a]	Observation time	Observed by	Object classes
Zartarian et al. 1998	2, 4 years	4	1 day	Video tape	Non-dietary objects
Groot et al. 1998	3-36 months	42	2.5 hours [b]	Parent	Pacifiers, toys intended for mouthing, other toys, fingers, non-pacifiers
Juberg et al. 2001	0-36 months	275	1 or 5 days [c]	Parent	Pacifiers, non-pacifiers
Kiss 2002	3-36 months	169	4 hours [d]	Trained observer	Pacifiers, teethers, soft plastic toys, anatomy, non-food objects, etc.

[a] Total number of subjects.
[b] Ten 15-minute observation periods over 2 days.
[c] A total of 107 subjects were observed for one day; 168 subjects were observed on 5 non-consecutive days.
[d] Twelve 20-minute observation periods over at least two days.

Groot et al. (1998) studied the mouthing behavior of 42 children between the ages of 3 and 36 months for the Dutch Consensus Group. In this study, parents observed and recorded mouthing activity during ten 15-minute periods over 2 days. Parents were given a questionnaire and stopwatch for recording observations along with video-taped instructions. Objects mouthed were classified as dummies (pacifiers), toys intended to be mouthed (i.e., teethers), other toys, fingers, or non-pacifiers (all objects, except pacifiers). Parents also recorded the time that the child was awake. Thus, daily mouthing times were estimated by multiplying the mouthing time (minutes per hour) by the time awake

(hours per day). Groot et al. found that pacifiers and fingers were the objects most frequently mouthed, and that the mean daily mouthing duration generally declined with age. Estimated daily mouthing times for four object categories are given in Table IX-5. The mean daily mouthing duration for pacifiers declined with age over all age groups. The mean daily mouthing durations for toys, teethers, and non-pacifiers were greatest in the 6 to 12-month-old group, then declined with increasing age.

Groot et al. also found considerable variability in mouthing duration. The standard deviations were roughly equal in magnitude to the mean mouthing durations, and the ranges were large. For example, in the 6 to 12-month-olds, the mean mouthing duration for toys was 22.1 minutes per day, with a standard deviation of 28.5 and a range of 0.4 to 101 minutes per day (Table IX-5). Many of the ranges in Table IX-5 have 0 minutes as the lower bound. Histograms of mouthing durations reveal highly skewed distributions with long tails (Greene 2002a).

Table IX-5. Estimated mouthing duration (minutes per day) in the Dutch Consensus Group Study (Groot et al. 1998). [a]

Age	N [b]	Object	Mean	SD	Range
3 – 6 months	5	Teether [c]	3.4	5.1	0 – 12.2
		Toy	11.3	10.0	0.6 – 26.8
		Non-pacifier	36.9	19.1	14.5 – 67.0
		Pacifier	94.9	NR	0 – 214.1
6 - 12 months	14	Teether	5.8	11.4	0 – 39.7
		Toy	22.1	28.5	0.4 – 101.5
		Non-pacifier	44.0	44.7	2.4 – 171.5
		Pacifier	27.3	NR	0 – 112.6
12 – 18 months	12	Teether	0.0	0.1	0 – 0.4
		Toy	3.6	3.5	0 – 10.4
		Non-pacifier	16.4	18.2	0 – 53.2
		Pacifier	17.3	NR	0 – 94.8
18 – 36 months	11	Teether	0.0	0.0	0 – 0
		Toy	1.1	1.2	0 – 3.8
		Non-pacifier	9.3	9.8	0 – 30.9
		Pacifier	20.8	NR	0 – 155.0

[a] Daily mouthing time was extrapolated from ten 15-minute observations over two days.
[b] N, number of children; NR, not reported; SD, standard deviation.
[c] Described by the authors as toys intended for mouthing.

The Groot et al. study was used to estimate daily DINP exposure in the Dutch Consensus Group (RIVM 1998) and CPSC (1998) risk assessments. The CPSC staff concluded that this study, while well-designed, was limited by the relatively small number of subjects. Furthermore, the study participants were a convenience sample whose relevance to the U.S. populations is uncertain (CPSC 1998).

Juberg et al. (2001) reported a larger observational study of mouthing behavior that was conducted in Western New York. In this study, parents observed their children for 1 or 5 days (Table IX-4). Thus, it was not necessary to extrapolate to obtain daily mouthing times. Parents were given a standard diary form with written instructions. Mouthing durations were recorded to the nearest minute. Objects mouthed were classified as pacifiers or non-pacifiers. The authors found that pacifiers were the most common objects mouthed and that there was considerable variability in mouthing duration.

In phase I (pilot) and phase II of the study, a total of 217 children ages 0 to 36 months were observed for 1 day. The mean mouthing duration for non-pacifiers was greater in the 0 to 18-month-old group (33 minutes per day) than in the 19 to 36-month-old group (5 minutes per day) (Table IX-6). The mean mouthing duration for pacifiers was roughly comparable in both groups. Variability in mouthing behavior was evident in the broad range of mouthing durations and in the large percentage of zero mouthing durations that were recorded. For example, among 0 to 18-month olds, 51 percent did not mouth pacifiers and 33 percent did not mouth non-pacifier objects. Reported daily mouthing durations in this age group ranged from 0 to 210 minutes per day for non-pacifiers and from 0 to 850 minutes (14 hours) per day for pacifiers. Histograms of mouthing durations revealed highly skewed distributions with long tails.

Table IX-6. Observed mouthing duration (minutes per day) in the study by Juberg et al. (2001)[a]

Age months	Days[b]	N	Object	Mean	SD	Median	% Zeros	Range
0 – 18	1	107	Non-pacifier	33	46	16	33	0 – 210
			Pacifier	108	187	0	51	0 – 850
19 - 36	1	110	Non-pacifier	5	14	0	72	0 – 95
			Pacifier	126	246	0	73	0 – 750
4 – 21	5[c]	793	Non-pacifier	36	52	16	17	0 – 338
	5	168	Non-pacifier	36	48	17	1.8	0 – 312

[a] Daily mouthing times were recorded by parents over 1 or 5 days.
[b] Days, number of days of observations; N, number of observations; NR, not reported; SD, standard deviation; number of zero mouthing durations.
[c] Observations were recorded on 5 non-consecutive days over 2-months (Juberg et al. 2001, Figure 3). Data were presented as either 1-day (n=793) or 5-day averages.

In phase III, 168 children ages 4 to 21 months (3 to 18 months at time of recruitment) were observed for 5 non-consecutive days over 2 months. When presented as 793, 1-day observations, the non-pacifier mouthing durations were roughly comparable to those for the 3 to 18-month-old group, except that the percentage of zeros was lower (17% vs. 33%) (Table IX-6). When presented as 168, 5-day averages, the mean, median, and standard deviation were about the same, but the percentage of zeros declined to about 2 percent. This suggests that most children who did not mouth non-pacifier objects during

1 day of observation were likely to mouth such objects if observed for a longer period of time.

Strengths of the Juberg et al. study are the relatively large numbers of children observed and that children were observed for up to 5 days. However, the study participants are a convenience sample. The CPSC staff considers that the use of untrained observers is a disadvantage. There were, in effect, 385 observers, which may introduce significant variability. Furthermore, the 1-day observation period is a significant concern, due to observer fatigue and attentiveness. Groot et al. expressed concern about observer fatigue in their study, in which observers (parents) were limited to two consecutive 15-minute observation periods (Groot et al. 1998). Mouthing durations were recorded to the nearest minute in the Juberg et al. study, whereas stopwatches were used by the Dutch Consensus Group (Groot et al. 1998) and CPSC (Greene 2002a; Kiss 2002) studies.

b. CPSC Observational Study

As part of the process of refining the 1998 risk assessment, the CPSC staff conducted a new observational study of children's mouthing activity (Greene 2002a; Kiss 2002). Study participants were recruited by random digit dialing in the Chicago and Houston metropolitan areas. Demographic information was collected, including race, income, education, and number of siblings. In phase I, the mouthing behavior of 491 children 0-to-6 years of age was observed and recorded by their parents or guardians. In phase II, a total of 169 children age 3 to 36 months were observed by trained observers for a total of 4 hours (12, 20-minute observations over 2 days). 109 of 169 children in phase II also participated in phase I. Objects mouthed were classified into 13 categories and subcategories, including pacifiers, non-pacifiers, soft plastic toys, soft plastic teethers and rattles, and other soft plastic items. Soft plastic toys are of interest, because many of these contain DINP (see above). Manufacturers have removed DINP from soft plastic teethers and rattles at the request of CPSC.

The exposure time, that is, the amount of time the children were awake and not eating, was recorded by the parents of 109 children in phase I. The average exposure time was related to the child's age. Thus, the average exposure time was estimated by the model:

$$Exposure = 9.46 + 0.0375 \times Age \qquad \text{(IX.2)}$$

where: *Exposure*, exposure duration in hours; and *Age*, child's age in months.

The standard deviation was 1.26 hours. Daily mouthing durations were estimated by multiplying hourly mouthing times by the exposure time.

The following results are from phase II observations only. As in previous studies, the items most frequently mouthed included pacifiers and fingers. All participants mouthed non-pacifier objects, about half mouthed soft plastic toys, and less than half mouthed soft plastic teethers and rattles (Table IX-7). The mean mouthing duration for non-pacifiers declined with age, ranging from 70 minutes per day in the 3 to 12-month-old group to 37

minutes per day among 24 to 36-month-olds. Although the age ranges differ, the mean non-pacifier mouthing duration appears to be somewhat greater in comparison to other studies. For example, the mean non-pacifier mouthing duration was 70 minutes per day among 3 to 12-month-olds in the CPSC study, 44 minutes per day among 6 to 12-month-olds in the Groot et al. study, and 33 minutes per day among 0 to 18-month-olds in the Juberg et al. study.

Mouthing of soft plastic teethers and rattles was greatest in the 3 to 12-month-old group (1.8 min/d), while mouthing of soft plastic toys was greatest in the 12 to 24-month-old group (1.9 min/d). Mouthing of all soft plastic articles is somewhat greater, but many of these other plastic products (e.g., cups, straws, eating utensils) are not made of PVC.

Table IX-7. Estimated daily mouthing duration (minutes per day) for selected objects in the CPSC observational study (Greene 2002a) [a]

Age	N [b]	Object mouthed	Mean	Median	95%	% Zeros [c]
3 – 12 months	54	Soft plastic toys	1.3	0.0	7.1	57
		Soft plastic teethers, rattles	1.8	0.0	12.2	70
		All soft plastic	4.4	1.2	17.5	22
		Non-soft plastic toys, teethers, rattles	17.4	12.6	58.0	6
		Pacifiers	33.0	0.0	187.4	57
		Non-pacifiers	70.1	65.6	134.4	0
12 – 24 months	66	Soft plastic toys	1.9	0.1	8.8	42
		Soft plastic teethers, rattles	0.2	0.0	0.9	91
		All soft plastic	3.8	2.2	13.0	12
		Non-soft plastic toys, teethers, rattles	5.7	3.2	18.6	8
		Pacifiers	26.6	0.0	188.5	73
		Non-pacifiers	47.4	37.0	121.5	0
24 – 36 months	49	Soft plastic toys	0.8	0.0	3.3	53
		Soft plastic teethers, rattles	0.2	0.0	0.8	14
		All soft plastic	4.2	1.5	18.5	27
		Non-soft plastic toys, teethers, rattles	2.2	0.8	10.7	98
		Pacifiers	18.7	0.0	136.5	90
		Non-pacifiers	37.0	23.8	124.3	0

[a] Data for soft plastic toys, all soft plastic, and non-pacifiers from (Greene 2002a, Tables 12a-c). Other data estimated as described in (Greene 2002a) (provided by Dr Greene).

[b] N, total number of children observed; 95%, 95th percentile value; % zeros, percentage of children not mouthing the object.

[c] From Greene 2002a, Table 9.

As with previous studies, there was considerable variability in mouthing duration. This is evidenced by standard deviations that are of similar magnitude to mean mouthing times, relatively low median values, and relatively high 95th percentile values. For some object/age groups, significant numbers of zero mouthing times were observed. Histograms (not shown) of mouthing duration revealed highly skewed distributions with long tails (Greene 2002a).

The CPSC observational study is similar in overall design to the study by Groot et al. (1998). However, the CPSC study differs in the use of a demographically representative random sample, trained observers, larger sample size (n=169), and a more detailed classification of the objects mouthed. These factors provide the ability to make reliable estimates of daily mouthing duration for specific object classes.

c. Summary of Observational Studies

To summarize, three observational studies of children's mouthing behavior are available that are suitable for use in exposure assessment (Greene 2002a; Groot et al. 1998; Juberg et al. 2001). These studies demonstrate that mouthing duration depends on the child's age and type of object mouthed. Mouthing activity generally declines with age. The objects most frequently mouthed are pacifiers and fingers. There is considerable variability in mouthing duration. Distributions of mouthing duration are highly skewed with long tails. On any given day, many children do not mouth particular object types, such as soft plastic toys or pacifiers. However, all children engage in mouthing activity.

3. Oral Exposure Assessment

a. Previous Risk Assessments

Several agencies have estimated oral exposure to DINP in children's products (Table IX-8). The Dutch Consensus Group (RIVM 1998) performed a risk assessment for the European Union. They used DINP migration data from a human subject study combined with mouthing duration data from Groot et al. (1998). They used Monte Carlo analysis (bootstrap procedure) to estimate exposure distributions for the use of teethers (toys intended to be mouthed) in children of various age groups. The mean (9.66 µg/kg-d) and 95th percentile (26.0 µg/kg-d) exposures were greatest in the 6 to 12-month-old age group. The *in vivo* migration data were likely more reliable than the *in vitro* data available at the time of this study. However, the *in vivo* data did not encompass a broad range of products, as only one teether and one standard disk were included. The use of mouthing data for "toys intended to be mouthed" may exclude other soft plastic (PVC) toys that children routinely mouth. While the Groot et al. data were the only mouthing data available at the time, they are based on a relatively small (n=42) sample size.

In the 1998 risk assessment, CPSC used migration data on 31 DINP-containing teethers and toys (CPSC 1998). Migration rates were scaled by multiplying them by the ratio of the migration rate with human subjects to the *in vitro* migration rate obtained by the impaction method. The ratio (39.5) was derived from the CPSC human subject study

using a toy with a relatively high migration rate (Chen 1998a). Thus, the inherent variability in migration rates among a series of products was maintained. To estimate mouthing duration, the CPSC staff reanalyzed the data from Groot et al. (1998), which the authors kindly provided. The mouthing times for "toys intended for mouthing" and "other toys" were combined to encompass products that could be made of PVC. Mouthing data were also divided into two age groups—3 to 12 and 13 to 26 months old. CPSC then estimated lognormal distributions of the mouthing duration, migration rate, and the *in vivo*: *in vitro* ratio (Greene 1998). Average and 95th percentile exposures were estimated by combining the lognormal distributions.

The estimated exposure was greatest in the 3 to 13-month old group, where the mean and 95th percentile exposures were 5.7 and 94.3 μg/kg-d, respectively. Although the 95th percentile exposure was below the ADI of 150 μg/kg-d, the CPSC staff concluded that there were significant uncertainties in the exposure assessment, and recommended additional work to conduct a more extensive observational study of mouthing activity and to develop a laboratory test method that better estimates the amount of phthalate released when children mouth DINP-containing products (CPSC 1998).

Recently, the CHAP (CPSC 2001) estimated oral DINP exposure using the upper bound (estimated 95th percentile value) migration rate obtained in the CPSC human subject study (Chen 1998a). The CHAP assumed mouthing durations of 3 hours and 1 hour per day for children 0 to 18 and 19 to 36 months old, respectively. Thus, upper bound measures of migration and exposure were combined to produce an upper bound estimate of oral exposure of 280 μg/kg-d in children 0 to 18 months old. However, the new migration (Chen 2002) and mouthing (Greene 2002a) data were not available at the time that the CHAP report was published.

The most recent European Union risk assessment used the maximum *in vivo* migration rate from the Dutch Consensus Group study (RIVM 1998) and assumed 3 hours of exposure per day (France 2001). This resulted in an upper bound exposure estimate of 200 μg/kg-d in children 3 to 12 months old.

b. Updated CPSC Risk Assessment

Methodology. The updated CPSC risk assessment used mouthing duration data from the CPSC observational study (Greene 2002a; Kiss 2002). Mouthing data included hourly mouthing times, as well as exposure times (time awake and not eating) (see above). Mouthing data were used to estimate the mouthing duration for children of different ages and for different product categories. Age ranges modeled were 3-to-12 months, 12-to-24 months, and 24-to-36 months. Product categories included:

- Soft plastic toys
- Soft plastic toys, teethers, and rattles
- All soft plastic objects
- All toys, teethers, and rattles
- Pacifiers

The categories "soft plastic toys" and "soft plastic toys, teethers, and rattles" are both subsets of the broader categories "all soft plastic objects" and "all toys, teethers, and rattles." Pacifiers are not a subset of any other category. Currently, DINP is used in soft plastic toys, but not in teethers, rattles, pacifiers, or toys made from other materials. Therefore, exposure calculations for product categories other than soft plastic toys are hypothetical.

Daily mouthing times were calculated by multiplying the hourly mouthing times (minutes per hour) for each age group and product category by the exposure time (hours per day). There were 54, 66, or 49 hourly mouthing times for children of age 3-12, 12-24, or 24-36 months, respectively. The exposure time was calculated by equation (IX.2), and had a standard deviation of 1.26 hours per day (Greene 2002b). Thus, the exposure time and, consequently, the daily mouthing time are dependent on the child's age in months. The equation and standard deviation were used to generate a table of 21 equally probable values for each age (by month), assuming a normal distribution (Greene 2002b). The 21 values were for the 5^{th}, 10^{th}, 15^{th} ... 95^{th} percentile exposure times, plus the 0.1^{th} and 99.9^{th} percentiles.

New DINP migration data for 24 soft plastic children's articles (primarily toys) were obtained using the JRC laboratory method (Chen 2002). Products were selected from the list of objects mouthed in the CPSC observational study (2002a). Thirty-six of the 85 (42%) soft plastic articles that were tested contained DINP (see above). To obtain migration rates disks must be cut from the article. As a result, migration rates could be obtained only from 24 articles, because other articles were either too small or solid, thereby preventing disks from being cut. To adjust for the prevalence of DINP (42%) in soft plastic toys, there would need to be (24/0.42=) 57 total objects of which (57-24=) 33 would not contain DINP. As a result, 33 zero migration rates were included with the set of 24 migration rates. This was the basic case that represents the best estimate of current oral exposure to DINP from children's products.

In contrast to the basic case, all hypothetical cases assumed 100% prevalence of DINP in articles rather than 42%. One such case simulated a scenario where all soft plastic toys contain DINP. Other cases assumed that DINP was in teethers and rattles in addition to soft plastic toys, in all soft plastic objects including straws and food contact items, and in pacifiers. These cases were based on the mouthing times associated with the appropriate category of object mouthed. In all these scenarios, it was assumed that the DINP migration rates would be the same as those for the soft plastic toys that were tested.

The DINP migration data were adjusted by an *in vivo*: *in vitro* ratio, as in the 1998 CPSC risk assessment (CPSC 1998). However, in this case, the mean ratio was approximately 0.33, rather than 39.5 as in the 1998 risk assessment, because the JRC method slightly over predicts the *in vivo* migration rate. The *in vivo* migration rate data were from the Dutch Consensus Group study using the standard disk (RIVM 1998). Raw data from the human subject study were kindly provided by Rinus Rijk, TNO. Although 20 subjects participated in the study, we excluded one outlier (Greene 2002b). The *in vitro* migration rate with the standard disk was measured by the CPSC Division of Chemistry (LSC),

which was done as part of the JRC interlaboratory study (Simoneau et al. 2001). The standard disk in the interlaboratory study is of the same formulation used in the Dutch human subject (i.e., *in vivo*) study, but is from a new batch. The new migration data with soft plastic toys were also from LSC (Chen 2002). The toy and standard disk migration rates were both measured by the JRC modification of the TNO method (Simoneau et al. 2001).

Body weights for children ages 0 to 36 months were obtained from published tables (EPA 1997, Table 7-1). Thus, body weights are dependent on the child's age in months. Body weights for males and females were averaged. Values for the 5^{th}, 10^{th}, 15^{th} ... 95^{th} percentiles of body weight for each age by month were obtained by interpolation of the tables, as described in Greene (2002b). This resulted in a table with 37 rows (0-36 months) by 19 columns (percentiles).

The following distributions were used to estimate exposure (Greene 2002b):

- Migration data on 24 articles obtained by the JRC laboratory method (Chen 2002). In the basic case only (soft plastic toys, 42% containing DINP), 33 zero migration rates were included to adjust for the prevalence of DINP. (Mean = 4.1 ± 2.7 μg/min for n=24).
- Migration data on the standard disk obtained with 19 subjects in the Dutch Consensus Group human subject study (RIVM 1998). (The study had 20 subjects; one outlier was removed.) (Mean = 1.17 ± 0.38 μg/min).
- Migration data on the standard disk obtained by the JRC method. The mean migration rate was 4.18 ± 0.45 μg/min (n=5).
- Hourly mouthing duration data (minutes per hour) from the CPSC observational study of 169 children aged 3 to 36 months (Greene 2002a). The sample size was 54, 66, or 49 for children of age 3-12, 12-24, or 24-36 months, respectively.
- Exposure time (hours per day) from the CPSC observational study (Greene 2002a). The mean is given by equation (IX.2). Exposure time is dependent on the child's age in months (n=21 per child).
- Body weight (EPA 1997, Table 7-1). Body weight is dependent on the child's age in months (n=19 per child).

The daily oral DINP intake was estimated by the equation:

$$E = \left[M_{product} \times \left(\frac{M_{human}}{M_{lab}} \right) \times T_{hour} \times T_{day} \right] \Big/ B \qquad \text{(IX.3)}$$

where: E, daily oral exposure, micrograms per kilogram per day (μg/kg-d); $M_{product}$, migration rate of the product as measured by the JRC method, μg/min; M_{human}, migration rate of the standard disk with human subjects, μg/min; M_{lab}, migration rate of the standard disk by the JRC laboratory method, μg/min; T_{hour}, hourly mouthing time, minutes per hour; T_{day}, exposure duration (time awake and not eating), hours per day; and B, body weight, kg. All migration rates are for a surface area of 10 cm^2.

Distributions of the daily DINP exposures were estimated by Monte Carlo methods (bootstrap procedure), as described by Greene (2002b). The bootstrap involves resampling from the data in a way that would be analogous to resampling from the population. The bootstrap produces not only point estimates for desired quantities such as mean, median and percentiles, but also the confidence intervals. It incorporates both the variability in the data and the uncertainty arising from sampling.

The procedure involved developing the probability distribution of DINP intake, which consists of all possible values for DINP intake and the probability that each value occurs. These were estimated from the various data sources including mouthing times, migration rates, body weight and the scaling factors (see above). The procedure was implemented in a manner that preserved the dependence of the mouthing time, exposure time, and body weight on age, and the independence of the other variables. Once the probability distribution is obtained, then desired statistics such as the mean, median, and percentiles can easily be computed. Confidence intervals were estimated by resampling the joint distribution with replacement 2,000 times (Greene 2002b).

Assumptions. The Monte Carlo procedure was implemented in a manner that preserved the dependence of the hourly mouthing duration, exposure duration, and body weight on age. The daily mouthing duration (product of the hourly mouthing duration and exposure time), product migration rate, human subject migration rate, and laboratory migration rate were assumed to be independent. There is no reason to believe that the latter subset of variables would be correlated. For example, children with longer mouthing times will not preferentially mouth toys with high migration rates.

Forty-two percent of the soft plastic articles (mainly soft plastic toys) tested contained DINP. Therefore, in calculating the exposure for the basic case (soft plastic toys, 42% containing DINP), we adjusted for the prevalence of DINP. This adjustment assumes that each time a child mouths a soft plastic toy, the probability of selecting a toy with DINP is 42 percent (i.e., 24/57).

In 1998, DINP was present in about 90 percent of the soft plastic toys and teethers tested by CPSC (Chen 1998). Currently, teethers, rattles, and pacifiers do not contain DINP. At the request of CPSC, manufacturers voluntarily removed DINP from teethers and rattles in 1999. A few pacifiers were made with phthalate-plasticized PVC, but these were voluntarily discontinued, as well. Clearly, not all soft plastic objects are made of PVC, and the category "all toys, teethers, and rattles" includes materials other than plastic. However, the use of PVC and DINP or other phthalates in children's products and other household products could change over time. Therefore, several hypothetical cases were included: soft plastic toys (100% containing DINP); teethers, and rattles; all soft plastic objects; all toys, teethers, and rattles; and pacifiers. One-hundred percent prevalence of DINP was assumed for all of the hypothetical cases. These are estimates of the exposures that could result if the use of DINP were to increase in the future.

The DINP migration data were obtained with a sample consisting primarily of soft plastic toys, that were selected from the list of objects mouthed in the observational study. This

risk assessment assumes that the toys tested accurately represent the universe of soft plastic toys. However, certain small or solid shapes, such as dolls or action figures, were not amenable to testing by the JRC method. In applying the migration data to other product categories (teethers, rattles, etc.), we are further assuming that the migration data would also represent the other product categories. In the 1998 CPSC risk assessment, there did not appear to be any difference in migration rate (by the impaction method) between soft plastic toys and teethers. However, the migration rates for a pacifier and bottle nipple containing diisooctyl phthalate were relatively high, in comparison to DINP-containing products (Chen1998a). Therefore, the estimated exposures for pacifiers must be interpreted with caution.

The JRC laboratory method gives migration rates in units of μg/10 cm^2/h. Thus, we are also assuming a constant surface area of 10 cm^2. CPSC previously assumed 11 cm^2 (CPSC 1983; CPSC 1998), which is an estimate of the approximate surface area mouthed by a child. There are no data to justify whether 10 or 11 cm^2 is more appropriate, and 10 cm^2 is consistent with other risk assessments (RIVM 1998; France 2001).

Results. The basic case—soft plastic toys, adjusting for the prevalence of DINP—represents the best estimate of current oral exposure to DINP in children's products. For the basic case, the greatest exposure was obtained with children aged 12-to-24 months (Greene 2002b). The estimated mean DINP exposure was 0.08 (95% confidence interval (CI) 0.04-0.14) μg/kg-d (Table IX-9). The median (50^{th} percentile) exposure was 0.00 (0.00-0.00) μg/kg-d. The 95^{th} percentile exposure was 0.53 (0.24-0.89) μg/kg-d (Table IX-9). The 99^{th} percentile exposure (not shown) was 1.5 (0.89-2.3) μg/kg-d.

The hypothetical cases represent the exposures that could result if the use of DINP in children's products were to increase in the future. For example, in 1998 about 90 percent of soft plastic teethers and toys contained DINP (Chen 1998a). No pacifiers contained DINP, although a small number contained diisooctyl phthalate. For the hypothetical cases, all assuming 100 percent prevalence of DINP, the estimated exposure generally increased in the order: soft plastic toys < soft plastic toys, teethers, and rattles < all soft plastic items < all toys, teethers, and rattles < pacifiers (Table IX-9). This reflects differences in mouthing durations (Table IX-7). For soft plastic toys (100% containing DINP), the greatest exposure was with children aged 12-to-24 months. The estimated mean DINP exposure was 0.22 (0.11-0.32) μg/kg-d (Table IX-9). The median (50^{th} percentile) exposure was 0.00 (0.00-0.05) μg/kg-d. The 95^{th} percentile exposure was 1.1 (0.62-1.6) μg/kg-d (Table IX-9).

For "all toys, teethers, and rattles," exposure was greatest among 3 to 12-month-olds. The estimated mean exposure was 2.9 μg/kg-d, while the median was 1.4 μg/kg-d. The estimated 95^{th} percentile exposure was 10.7 (6.5-16.1) μg/kg-d.

As with previous studies, the greatest estimated exposures were obtained with pacifiers, due to the relatively long mouthing duration. Exposure was greatest among 3 to 12-month-olds. The estimated mean exposure was 4.8 μg/kg-d, while the median was 0.00 (0.0-0.64) μg/kg-d (Table IX-9). That the median was zero reflects the observation that

57 percent of children did not mouth pacifiers (Table IX-7). The 95th percentile exposure was 24.6 (11.7-41.4) μg/kg-d (Table IX-9). The estimated 99th percentile exposure (not shown) was 62.4 μg/kg-d (28.4-101.5) (Greene 2002b).

Previously, CPSC (1998) estimated a mean oral exposure of 5.7 μg/kg-d for 3 to 12-month-olds mouthing teethers and toys, with a 95th percentile of 94.3 μg/kg-d (Table IX-8). The mean is about double the estimated exposure from 3 to 12-month-old children mouthing "all toys, teethers, and rattles" in the updated risk assessment, with a mean of 2.9 μg/kg-d and 95th percentile of 10.7 μg/kg-d (Table IX-9). For 13 to 26-month-olds, CPSC previously estimated an oral exposure of 0.7 μg/kg-d for teethers and toys (95th percentile 7.6 μg/kg-d). This is in reasonably good agreement with the present value for 12 to 24-month-olds mouthing all teethers, toys, and rattles, with a mean of 0.84 μg/kg-d and 95th percentile value of 3.4 μg/kg-d.

The estimated oral exposures in the updated risk assessment are lower than other reported estimates (compare Table IX-8). There are several differences in methodology and input data that may help to explain the differences. Both CPSC risk assessments used migration rates from a range of products, 31 in 1998 and 24 in the present case. Several other risk assessments relied on human subject data with only one or two products. The Dutch (RIVM 1998) and Canadian (Health Canada 1998) risk assessments used migration rates from the Dutch human subject study, which used a standard PVC disk and one teether. Furthermore, the CHAP (CPSC 2000) and European Union (France 2001) used upper bound migration rates from human subject studies.

The present risk assessment has somewhat lower migration rates than the 1998 CPSC risk assessment. The migration rates from the 1998 risk assessment, obtained by impaction, can be made comparable to the present results if they are multiplied by the 39.5-fold adjustment factor and converted to units of μg/10 cm^2/min. Thus, the mean migration rate in 1998 of 8.2 μg/11 cm^2/h (Chen 1998a) becomes 4.9 μg/10 cm^2/min. This is slightly greater than the present mean of 4.1 μg/10 cm^2/min. The 95th percentile migration rate in 1998 of 29.7 μg/11 cm^2/h is equivalent to 17.8 μg/10 cm^2/min, which is also greater than the current 95th percentile of 10.2 μg/10 cm^2/min.

Different estimates of mouthing duration also lead to different exposure estimates. The CHAP (CPSC 2001) and European Union (France 2001) assumed mouthing durations of 1 to 3 hours per day, for the purpose of making upper bound estimates of exposure. These mouthing durations are not directly derived from empirical data. They are the same values used by CPSC in the 1983 risk assessment for DEHP (CPSC 1983).

Differences in mouthing duration may be attributed to differences in the methodology used in the observation studies, which are discussed in detail above (section IX.A.2). The most significant difference is in the classification of objects mouthed. Juberg et al. (2000) classified objects as pacifiers or non-pacifiers. However, non-pacifiers include anatomy (fingers and hands) and food contact items, which are not PVC (Greene 2002a; Juberg et al. 2000; Kiss 2002). The Dutch Consensus Group study classified objects into more specific categories, such as pacifiers, teethers, and toys (Groot et al. 1998).

However, CPSC classified objects into the most specific categories, such as soft plastic toys. Thus, in comparing the 1998 CPSC risk assessment, which used mouthing data from Groot et al., with the present risk assessment, it is not surprising that the 1998 estimates for "teethers and toys" are closest to the present estimates for "all toys, teethers, and rattles" than to "soft plastic toys" or "soft plastic toys, teethers, and rattles" (see above).

It is also significant that in the basic case we adjusted for the prevalence of DINP. No other risk assessments made this adjustment. However, in 1998 about 90 percent of soft plastic toys and teethers contained DINP (Chen 1998a).

Other differences between the CPSC observation study and previous studies include the use of a demographically representative random sample, trained observers, and relatively large sample size (n=169).

Table IX-8. Estimates of oral DINP exposure (μg/kg-d) from children's products [a, b]

Agency	Product(s)	Age	Mean	Median	95%	Range	Reference
Dutch Consensus Group [c, d]	Teethers	3 - 6 months	9.66	7.17	26	– 70.7	RIVM 1998
		6 - 12 months	7.79	4.8	25.5	– 142	
		12 - 18 months	2.33	1.06	10.5	– 51.1	
		18 - 36 months	1.13	0.521	4.32	– 23	
Health Canada [d]	Teethers, toys	3 - 12 months	44	--	--	4 – 320	Health Canada 1998
		13 - 26 months	39	--	--	5 – 228	
	Pacifiers	3 - 12 months	120	--	--	18 – 640	
		13 - 26 months	62	--	--	5 – 458	
CPSC [e]	Teethers, toys	3 - 12 months	5.7	--	94.3	--	CPSC 1998
		13 - 26 months	0.7	--	7.6	--	
Austrian Standards Institute	Teethers	--	31.25	--	--	--	Fiala et al. 2000
Chronic Hazard Advisory Panel [f]	Toys	0 - 18 months	--	--	280	--	CPSC 2001
		19 - 36 months	--	--	66	--	
France [c, g]	Toys	3 – 12 months	--	--	200	31 – 226	France 2001

[a] Adapted from CPSC 2001.

[b] All units are in micrograms per kilogram per day.

[c] For the European Union.

[d] Based on the migration rates measured with human subjects by the Dutch consensus group, 5th percentile body weights, and assuming mouthing times of 1 to 3 hours for teethers and toys and 2 to 6 hours for pacifiers.

[e] The migration rate by the impaction method was multiplied by a scaling factor to adjust for the difference between impaction and human subjects.

[f] Estimate for "relatively highly exposed children" based on the estimated 95th percentile migration with human subjects (CPSC 1998) and assuming mouthing durations of 3 hours/day for 0 – 18-month-olds and 1 hour/day for 19 – 36-month-olds.

[g] Based on the maximum *in vivo* migration rate (8.9 μg/cm^2/min) in the Dutch Consensus Group study (RIVM 1998), 10 cm^2 surface area, 3 hour exposure duration, and 8 kg body weight (France 2001, Table 4.1.1.2.1). The low value in the range is based on the average migration rate in Steiner et al. 1998. The high value is the 95 percent upper confidence interval of the 95th percentile exposure, as calculated by CPSC (1998).

Table IX-9. Estimated oral exposure (μg/kg-d) to DINP in children's products [a]

Product	Age (months)	Mean [b]	Median	95th Percentile
Basic Case [c]				
Soft plastic toys (42% with DINP)	3-12	0.07 (0.03 – 0.13)	0.00 (0.00 – 0.00)	0.44 (0.15 – 0.82)
	12-24	0.08 (0.04 – 0.14)	0.00 (0.00 – 0.00)	0.53 (0.24 – 0.89)
	24-36	0.03 (0.01 – 0.06)	0.00 (0.00 – 0.00)	0.12 (0.04 – 0.23)
Hypothetical Cases [c]				
Soft plastic toys (100% with DINP)	3-12	0.17 (0.08 – 0.29)	0.00 (0.00 – 0.02)	0.94 (0.47 – 1.5)
	12-24	0.22 (0.11 – 0.32)	0.01 (0.00 – 0.05)	1.1 (0.62 – 1.6)
	24-36	0.07 (0.02 – 0.14)	0.00 (0.00 – 0.01)	0.27 (0.12 – 0.72)
Soft plastic toys, teethers, & rattles (100% with DINP)	3-12	0.45 (0.24 – 0.74)	0.05 (0.00 – 0.17)	2.2 (1.2 – 3.5)
	12-24	0.22 (0.12 – 0.34)	0.01 (0.00 – 0.06)	1.1 (0.64 – 1.7)
	24-36	0.08 (0.02 – 0.18)	0.00 (0.00 – 0.01)	0.33 (0.12 – 1.1)
All soft plastic items (100% with DINP)	3-12	0.63 (0.38 – 1.0)	0.12 (0.04 – 0.33)	2.9 (1.8 – 4.4)
	12-24	0.41 (0.26 – 0.60)	0.15 (0.07 – 0.27)	1.7 (1.1 – 2.5)
	24-36	0.37 (0.19 – 0.59)	0.08 (0.01 – 0.18)	1.7 (0.87 – 2.7)
All toys, teethers, & rattles (100% with DINP)	3-12	2.9 (1.8 – 4.3)	1.4 (0.87 – 2.3)	10.7 (6.5 – 16.1)
	12-24	0.84 (0.52 – 1.2)	0.33 (0.18 – 0.55)	3.4 (2.1 – 5.0)
	24-36	0.28 (0.15 – 0.44)	0.08 (0.04 – 0.14)	1.2 (0.59 – 2.1)
Pacifiers (100% with DINP)	3-12	4.8 (2.2 – 8.0)	0.00 (0.00 – 0.64)	24.6 (11.7 – 41.4)
	12-24	2.8 (1.2 – 5.0)	0.00 (0.00 – 0.00)	17.4 (6.4 – 30.8)
	24-36	1.7 (0.07 – 4.3)	0.00 (0.00 – 0.00)	5.4 (0.00 – 31.4)

[a] From Greene (2002b).
[b] Numbers in parentheses are 95% confidence intervals.
[c] See text.

B. Dermal Exposure

1. Estimates of Dermal Intake

Risk assessments of DINP exposure from children's products such as teethers and toys have generally considered only oral exposure (CPSC 1998; Health Canada 1998; RIVM 1998; Wilkinson and Lamb 1999). However, the CPSC risk assessment of DEHP exposure from children's products considered dermal exposure from products such as playpens and baby pants, as well as oral exposure (CPSC 1983). Recently, the CHAP (CPSC 2001) and the European Union risk assessment (France 2001) also considered dermal DINP exposure from PVC products such as rainwear, footwear, gloves, and toys.

To estimate dermal exposure to DEHP, CPSC scrubbed PVC products with a wood block covered with lanolin-impregnated cotton cloth (CPSC 1983). CPSC also assumed that 10 percent of the DEHP migrating to the skin would be absorbed. By this method, CPSC estimated that the dermal intake (i.e., absorbed dose) from vinyl playpens would be 96 mg DEHP per year, or roughly 33 μg/kg-d (CPSC 1983). CPSC also estimated a dermal dose from vinyl baby pants of 120 mg over 2 years, or roughly 21 μg/kg-d. Percutaneous absorption studies published since the 1983 risk assessment suggest that percutaneous absorption of DEHP is about 5 percent over a 5-day period (Elsisi et al. 1989). Thus, the dermal dose from DEHP is probably lower than what CPSC estimated.

France considered dermal exposure in its risk assessment for the European Union (France 2001). Deisinger et al. (1988) studied the percutaneous absorption of DEHP in rats from a PVC film containing 40 percent DEHP. DEHP was absorbed at a rate of 0.24 $\mu g/cm^2$-h. However, in the risk assessment, it was assumed that DINP would be absorbed more slowly than DEHP (Elsisi et al. 1989). It was further assumed that an 8 kg child would handle soft plastic toys for 3 hours per day, and that the surface area contacting the toys would be 100 cm^2. The dermal intake was estimated to be 1 μg/kg-d. They also estimated that the intake for adults wearing PVC gloves for two hours per day, with a surface area (both hands) of 840 cm^2, would be 0.7 μg/kg-d.

The CHAP used two methods to estimate dermal exposure and absorbed dose (CPSC 2001, Appendix A). They used the percutaneous absorption rate from the study with DEHP-containing PVC film (Deisinger et al. 1998), and assumed that the absorption of DINP would be no greater than the absorption of DEHP. Using this method, which the CHAP termed the CF (Contact-Flux) method, the CHAP estimated dermal doses for children (19 to 36 months) and adults using rainwear of 3.2 to 3.9 μg/kg-d, respectively. The CHAP also estimated exposure from vinyl sandals of 14 μg/kg-d in children (19 to 36 months) and 3.9 μg/kg-d in adults, assuming that significant absorption would only occur through the dorsal surface of the foot. However, as noted by the CHAP, two samples of sandals tested by the CPSC Chemistry Laboratory contained DEHP and dibutyl phthalates, rather than DINP.

The CHAP also estimated that absorption of DINP through the oral mucosa during mouthing would range from 0.26 to 3.1 μg/kg-d, which they concluded was negligible in

comparison to oral exposure by ingestion (see also part IX.A.1.b, Studies with Human Subjects, above).

The CHAP used another method termed the AC (aqueous-clearance) method. In this method, the 95 percent upper confidence limit of the DINP migration in the CPSC human subjects study was used to estimate the release of DINP from PVC in contact with the skin. In some cases, such as absorption by the oral mucosa or the use of PVC sandals, the environment may be moist (due to the presence of saliva or perspiration) and mechanical action (either chewing or walking) may contribute to DINP release. These conditions would be more extreme than simply holding a PVC film in contact with the skin, as in the Deisinger et al. study. Percutaneous absorption was estimated by means of an empirically based model developed to estimate effective permeability constants for compounds in dilute aqueous solutions. By this method, the CHAP estimated an effective permeability constant (K_p^{eff}) of 2.4 cm/h for DINP.

Table IX-10. Estimates of dermal intake (µg/kg-d) of dialkyl phthalates from household products

Agency	Product	Age	Exposure (µg/kg-d)	Methodology	Reference
CPSC (DEHP)	Playpen liner Baby pants	Child Child	33 [a] 21 [b]	Migration estimated by scrubbing; assumes 10% dermal absorption	CPSC 1983
CHAP	Rainwear Sandals [c]	19-36 months Adult 19-36 months Adult	3.2 0.45 14 3.9	Migration and dermal absorption from Deisinger et al. (1998)	CPSC 2001, Appendix A
	Rainwear Sandals [c]	19-36 months Adult 19-36 months Adult	79 11 340 98	Migration from human subjects study (Chen 1998). Dermal absorption modeled (Bogen 1994).	
European Union	Soft plastic toys Vinyl gloves	Child Adult	1 0.7	Migration and dermal absorption from Deisinger et al. (1998)	France 2001

[a] CPSC estimated a dermal intake of 96 mg DEHP per year. Converted to µg/kg-d by assuming an 8 kg child.

[b] CPSC estimated a dermal intake of 120 mg DEHP in two years. Converted to µg/kg-d by assuming an 8 kg child.

[c] For absorption through the dorsal surface of the foot only.

The CHAP estimated greater dermal doses by the AC method. The dermal intakes for children (19 to 36 months) and adults using rainwear were estimated to be 79 or 11 µg/kg-d, respectively. The CHAP also estimated dermal doses from vinyl sandals of 340 µg/kg-d in children (19 to 36 months) and 98 µg/kg-d in adults. The CHAP also estimated that the absorption of DINP through the oral mucosa during mouthing would range from 15 to 210 µg/kg-d.

2. Methods for Estimating Dermal Intake

The CHAP used two methods of estimating dermal exposure and percutaneous absorption of DINP that yielded different results. The CF method is essentially similar to the method used in the European Union risk assessment (France 2001). The CF method is based on a study in which DEHP-plasticized PVC film is placed on the backs of rats (Deisinger et al. 1998). Thus, both migration and percutaneous absorption are measured in a single *in vivo* experiment. A similar approach has been used to estimate bioavailability of flame retardant chemicals (Maibach 1979; Ulsamer et al. 1978) and pesticides (Wester et al. 1996) in treated textiles. DEHP is another dialkyl phthalate with a slightly lower molecular weight (390 vs. 418) and log K_{ow} (7.45 vs. ≈9) (IARC 2000). The percutaneous absorption of DINP is expected to be roughly similar to that of DEHP (CPSC 2001; France 2001).

The principal advantage of this method is that it is based on empirical data. The relatively low percutaneous absorption observed by Deisinger et al. is consistent with other *in vivo* (Elsisi et al. 1989; Stoltz and El-hawari1983; Stoltz et al. 1985) and *in vitro* (Barber et al. 1992; Scott et al. 1987) studies of the percutaneous absorption of dialkyl phthalates. In addition, rodent skin is up to 10-fold more permeable than human skin (Wester and Maibach 1983). Therefore, the use of animal data may lead to overestimates of percutaneous absorption.

The principal disadvantage of this method is that it may not adequately model exposure under all conditions. For example, exposure to DINP in footwear may involve a moist environment and friction, which may lead to increased migration and percutaneous absorption (CPSC 2001; Maibach 1979; Ulsamer et al. 1978; Wester and Maibach 1983).

The AC method used the estimated 95th percentile DINP migration rate from the CPSC human subjects study as an upper bound estimate of migration. Percutaneous absorption was estimated by means of an empirically derived model developed for dilute aqueous solutions (Bogen 1994). However, this model was based on data with compounds having log K_{ow} values up to 4.11 and molecular weights up to 197.5. A similar type of model was based on data with compounds having log K_{ow} values up to 6 (Potts and Guy 1992). However, DINP and DEHP have exceptionally high log K_{ow} values (>7) and relatively high molecular weights. The models predict high permeability coefficients, based on the high log K_{ow} values. These are not consistent with the low absorption rates measured *in vivo* (Elsisi et al. 1989; Stoltz and El-hawari 1983; Stoltz et al. 1985; Wester et al. 1998) and low permeability constants measure *in vitro* (Barber et al. 1992; Scott et al. 1987). Furthermore, it is likely that intermediate values of log K_{ow} favor absorption, because the skin includes both hydrophobic and hydrophilic barriers.

For example, the CHAP calculated a K_p^{eff} value of 2.4 cm/h for DINP using the model (CPSC 2001, page 131, equation 2). Applying the same model gives a K_p^{eff} of 0.52 for DEHP. A permeability coefficient (K_p) of 0.57×10^{-5} cm/h was measured for DEHP using human skin *in vitro* (Scott et al. 1987). Thus the empircally determined K_p value for DEHP was orders of magnitude lower than the value calculated by the model. Even if

the *in vitro* methodology is an underestimate of the *in vivo* K_p, which may occur for hydrophobic compounds, the true rate of percutaneous absorption is likely to be much less than predicted by the model.

The percutaneous absorption of DINP and DEHP were measured using the pure compounds. Dissolving DINP or DEHP in water probably would favor absorption, due to partitioning between the vehicle and the skin (Wester and Maibach 1983). However, it would not be practical to measure absorption from aqueous solutions, because DINP and DEHP are practically insoluble; the solubility of DINP is less than 1 microgram per liter (µg/L). Furthermore, the concentration of DINP in saliva was estimated to be approximately 1 microgram per milliliter (µg/mL) (see above). Thus, it was likely present as a suspension, rather then as a solution. This tends to support the use of the empirical data using the pure compound, rather than the model for dilute aqueous solutions.

Therefore, the CPSC staff favors the use of empirical data (i.e., Deisinger et al. 1998; Stoltz and El-hawari 1983; Stoltz et al. 1985), rather than the mathematical model, to estimate percutaneous absorption. The mathematical model may not be applicable to extremely hydrophobic compounds such as DINP. Furthermore, the use of empirical data from well-designed and executed studies is preferred over mathematical models (CPSC 1992). The use of animal studies may tend to over predict percutaneous absorption in humans. However, the conditions of the empirical studies may not adequately model certain conditions of human exposure, such as from footwear or absorption by the oral mucosa, which may tend to increase migration and absorption. Therefore, the use of animals to estimate dermal exposure and bioavailability and the effects of actual use conditions on exposure and bioavailability remain as sources of uncertainty in estimating dermal exposure and risk.

C. Background Exposure to Dialkyl Phthalates

Dialkyl phthalates are used in many different products made from PVC and other plastics, including vinyl flooring, building materials, automobile interiors, medical devices, and children's products. DINP is used in vinyl flooring, wire and cable, stationery, wood veneer, coated fabrics, gloves, soft plastic toys, tubing, artificial leather, shoes, sealants, and carpet backing (CERHR 2000a). DINP is the principal dialkyl phthalate used in soft plastic toys (Chen 1998a, 2002; Health Canada 1998; Marin et al. 1998; Rastogi 1998; Rastogi et al. 1997; Simoneau et al. 2001; Sugita et al. 2001; Vikelsøe et a. 1997). DINP and other dialkyl phthalates have not been used in teethers, rattles, and pacifiers in the U.S. since 1998.

Dialkyl phthalates—including DEHP, dibutyl phthalate, and butyl benzyl phthalate—may be found in water (ATSDR 1993; CERHR 2000b; Yin and Su 1996), ambient air (ATSDR 1993; CERHR 2000b), indoor air (CERHR 2000b; Ølie et al. 1997; Tienpont et al. 2000; Wechsler et al. 1984), and soil (ATSDR 1993). Dialkyl phthalates may also be found in food (ATSDR 1993; Giam and Wong 1987; MAFF 1996a; Tsumura et al. 2001;

Yin and Su 1996), infant formula (Baczynskyj 1996; MAFF 1996b), and parenteral nutrition products (Kambia et al. 2001).

DINP has rarely been reported in food or the environment and, when present, it is at very low levels (CERHR 2000a). DINP was detected at low, non-quantifiable levels in infant formula (MAFF 1996b) and in hospital food (Tsumura et al. 2001). DINP also was detected in indoor air at levels up to 20 ng/m^3 (Tienpont et al. 2000; Wechsler et al. 1984), and was a minor component in some samples of sedimented dust in residences (Ølie et al. 1997).

Food is believed to be the primary source of exposure to dialkyl phthalates (ATSDR 1993; CERHR 2000b). Dialkyl phthalates are not generally used in food packaging in the U.S. The primary source of the dialkyl phthalates in food is believed to be general environmental contamination, rather than food packaging (ATSDR 1993; MAFF 1996a,b). The average exposure to DEHP was estimated as 0.27 mg/person/day (ATSDR 1993), which is roughly equivalent to 3.8 µg/kg-d (Table IX-11). Total intake of DEHP in Canada from all exposure routes was estimated to range from 8 to 19 µg/kg-d for various age groups, with the greatest exposure in the 0.5 to 4-year-old group (Meek and Chan 1994). The average dietary intake of total dialkyl phthalates in the U.K. was estimated to range from 0.1 to 0.8 mg/person/day (MAFF 1996a), or 1-to-11 µg/kg-d. Tsumura et al. estimated the dietary intake of dialkyl phthalates in hospital food to be 519 µg/d (~7.4 µg/kg-d) DEHP, 65 µg/d (0.93 µg/kg-d) DINP, and 4.7 µg/d (~0.067 µg/kg-d) butyl benzyl phthalate (Tsumura et al. 2001). Huber et al. (1996) estimated that total DEHP exposure from all media would be no greater than 30 µg/kg-d.

Few data on background dialkyl phthalate exposure in infants are available. A Canadian study estimated the average DEHP exposure to be 19 µg/kg-d in 0.5 to 4-year-old children, based on a food basket survey (Meek and Chan 1994). In a study by the U.K. Ministry of Agriculture, Fisheries, and Food (MAFF), total DAP levels in unreconstituted infant formulae ranged from 1.2 to 10.2 µg/g (MAFF 1996b). Based on these levels, the average intake of total dialkyl phthalates from infant formula was estimated to be 130 µg/kg-d at birth and 100 µg/kg-d at 6 months of age (MAFF 1996b). However, infant formula in the U.S. appears to have lower dialkyl phthalate levels (Baczynski 1996). Total dialkyl phthalate levels ranged from 0.011 to 0.051 µg/g in ready to use formula and from 0.007 to 0.032 µg/g in powdered formula (Baczynski 1996). These levels roughly correspond to exposures ranging from 0.05 to 5 µg/kg-d in 5 to 12-month-old infants.* An estimate of the dietary intake of total phthalates in infants in Europe was reported to be 23 µg/kg-d (Janssen et al. 1998).

Exposure to DEHP has been reported in patients undergoing medical procedures such as IV therapy, enteral and parenteral nutrition support, blood transfusion, hemodialysis and peritoneal dialysis, cardiopulmonary bypass (CPB) and extracorporeal membrane

* Assumes 125 grams of powder per quart (1.14 liters) of reconstituted formula, which is typical of manufacturers' instructions. Also assumes 3 to 4 feedings per day at 210 to 240 mL per feeding (Nelson et al., 1996, p. 162) and a body weight of 10 kg.

oxygenation (ECMO) (Barry et al. 1989; CERHR 2000b; FDA 2001). For example, DEHP and its metabolites have been detected in the blood of infants undergoing tranfusions (Plonait et al. 1993; Sjober et al. 1985a,b) or extracorporeal membrane oxygenation (Karle et al. 1997). Estimated DEHP exposures may be as high as 8.5 mg/kg-d in adult trauma patients and 22.6 mg/kg in neonates undergoing exchange transfusions (FDA 2001).

Colon et al. (2000) measured phthalate levels in serum samples from 41 girls under age 8 with premature breast development (thelarche). In 68 percent of patients, levels of dimethyl, diethyl, dibutyl, di-(2-ethylhexyl), and mono-2-ethylhexyl phthalates were at levels greater than in the controls.

Table IX-11. Estimated background exposures to phthalates

Dialkyl phthalate	Exposure (µg/kg-d)	95% [a] (µg/kg-d)	Based on	Reference
Adults				
DEHP	0.60	3.05	Urinary metabolites	David 2000
	0.71	3.6	Urinary metabolites	Kohn et al. 2000
	3.8	--	All routes	ATSDR 1993
	7.4	--	Hospital food	Tsumura et al. 2001
	8-19	--	All routes	Meek and Chan 1994
	≤30	--	All routes	Huber et al. 1996
DINP	0.21	1.08	Urinary metabolites	David 2000
	ND	1.7	Urinary metabolites	Kohn et al. 2000
	0.93	--	Hospital food	Tsumura et al. 2001
Total phthalates	1 - 11		Diet	MAFF 1996a
	8.4	--	Hospital food	Tsumura et al. 2001
	15.1	--	Urinary metabolites	Kohn et al. 2000
	15.4	--	Urinary metabolites	David 2000
Children				
DEHP	19	--	All routes [b]	Meek and Chan 1994
Total phthalates	0.05 – 5.0	--	Infant formula [c]	CPSC 1998
	23	--	Diet	Janssen et al. 1998
	100 – 130	--	Infant formula [d]	MAFF 1996b

[a] 95%, 95th percentile value, where reported.
[b] For 0.5 to 4-year-olds.
[c] Estimated from the levels in infant formula in the U.S. (Baczynski 1996).
[d] Estimated from the levels in infant formula in the U.K.

As part of the *National Report on Human Exposure to Environmental Chemicals*, the Centers for Disease Control (CDC) measured urinary monoalkyl levels in participants 6 years of age and up (CDC 2001). Samples were collected in 1999. Daily intake levels of dialkyl phthalates were not estimated. The highest urinary levels were for monoethyl and monobutyl phthalates. Levels of mono-2-ethylhexyl phthalate (MEHP) were relatively low. The median mono(isononyl) phthalate (MINP) level was below the limit of detection.

Recently, Brock et al. (2002) measured monoester levels in the urine of 19 infants ranging from 11 to 17 months of age. Samples were collected in early 2000. Mean urinary monophthalate levels in the infants were higher than the median levels previously found in adults (Blount et al. 2000). Daily intake levels of dialkyl phthalates were not estimated. As with the adults, the highest urinary levels were for monoethyl and monobutyl phthalates. Levels of MEHP were relatively low. MINP was below the limit of detection.

Blount et al. (2000) measured urinary monoalkyl phthalate levels in 289 adults, which were a subset of the Third National Health and Nutrition Examination Survey (NHANES III). Monoesters with the highest levels were monoethyl, monobutyl, and monobenzyl phthalates, which were attributed to exposure to diethyl, dibutyl, and benzyl butyl phthalates. Women from 20 to 40 years of age had significantly higher levels of monobutyl phthalate, which may be due to the use of dibutyl phthalate in cosmetics (see also Stock et al. 2001). Other monoesters detected at lower levels 2-ethylhexyl, cyclohexyl, isononyl, and octyl phthalates. The low urinary levels of MEHP and MINP could be due to low exposures and/or to partial excretion in feces. Furthermore, only one MINP isomer (mono-3-methyl-5-dimethylhexyl phthalate) was measured, although DINP is a complex mixture of isomers.

Using the data on urinary metabolites by Blount et al. (2000), Kohn et al. (2000) estimated the daily exposure to dialkyl phthalates. They estimated median exposures as high as 8.5 µg/kg-d for diethyl phthalate. The median exposure for DEHP was estimated to be 0.71 µg/kg-d, with DINP below the detection limit. The 95th percentile DINP exposure was estimated to be 1.7 µg/kg-d. The total median exposure to the seven phthalates assayed was 15.1 µg/kg-d.

David (2001) also estimated exposure from the data of Blount et al. The MAFF measured the levels of urinary monesters in volunteers given known amounts of dialkyl phthalates (Anderson et al. 2000). Using the data of Anderson et al. and Blount et al., David estimated geometric mean exposures of 12.3 µg/kg-d for diethyl phthalate, 0.60 µg/kg-d for DEHP, and 0.21 µg/kg-d for DINP. The 95th percentile exposure for DINP was estimated to be 1.08 µg/kg-d. The total geometric mean exposure for the seven phthalates assayed was 15.4 µg/kg-d.

X. Risk Characterization

Estimated average and 95th percentile oral exposures for all cases and all object classes were below the ADI value of 120 μg/kg-d. For the basic case of soft plastic toys (42% containing DINP), the estimated mean exposure in 12 to 24-month-olds was 0.08 μg/kg-d (Table IX-9). The median exposure was 0.0 μg/kg-d, while the 95th percentile was 0.53 μg/kg-d.

For the hypothetical cases (assuming that 100% contain DINP), the estimated exposures generally increased in the order: soft plastic toys < soft plastic toys, teethers, and rattles < all soft plastic objects < all toys, teethers, and rattles < pacifiers. For soft plastic toys (assuming 100% contain DINP), the estimated mean exposure in 3 to 12-month-olds was 0.17 μg/kg-d (Table IX-9). The median exposure was 0.0 μg/kg-d, because 57 percent of children did not mouth soft plastic toys (Table IX-7), while the 95th percentile was 0.94 μg/kg-d.

The estimated exposures for "all toys, teethers, and rattles," were higher than all other categories, except pacifiers (Table IX-9). For this object class, the highest exposures were found in the 3-to-12 month age group. The mean exposure was 2.9 μg/kg-d, with a 95th percentile value of 10.7 μg/kg-d (95% CI 6.5 – 16.1). Thus, even the 95th upper confidence limit of the 95th percentile of exposure is below the ADI value.

No data on the health effects of combined exposures to dialkyl phthalates are available. Nonetheless, the 1998 Dutch Consensus Group risk assessment added the background dialkyl phthalate exposure to the estimated DINP exposure (RIVM 1998). This approach assumes that the health effects from dialkyl phthalates are additive, dialkyl phthalates induce similar health effects, act by similar mechanisms, and that they are essentially equipotent. While there are no data to support these assumptions, it may be considered to be a reasonable default assumption (CPSC 2001). One of the most common phthalates present in food is DEHP, which induces many of the same health effects as DINP, including spongiosis hepatis (David et al. 2000).

Janssen et al. (1998) estimated that the background exposure to total phthalates in children is about 23 μg/kg-d, which is greater than the mean and median exposures to DINP from mouthing "all toys, teethers, and rattles" (compare Tables IX-9). Assuming additivity of total phthalate exposure, the estimated background exposure of 23 μg/kg-d can be added to oral exposure estimates. Adding 23 μg/kg-d to the estimated oral exposure for "all teethers, toys, and rattles" is still below the ADI. For example, the 95 percent upper confidence limit of the 95th percentile exposure for children age 12 to 24 months would increase from 16.1 to 39 μg/kg-d, which is below the ADI of 120 μg/kg-d.

The greatest estimated oral exposures were found with the hypothetical case pacifiers, which is due to the comparatively long mouthing durations. Mouthing of pacifiers was greatest in the 3 to 12-month old age group. The estimated mean oral exposure was 4.8 μg/kg-d (Table IX-9). The median was zero, because 57 percent of the children in this age group did not mouth pacifiers (Table IX-7). The 95th percentile exposure was 24.6

(11.7-41.4) μg/kg-d (Table IX-9). For example, the best estimate of the 95th percentile exposure (24.6 μg/kg-d), as well as the 95 percent upper confidence limit of the 95th percentile exposure (41.4 μg/kg-d), are below the ADI of 120 μg/kg-d. The 99th percentile exposure (not shown in Table IX-9) was 62.4 μg/kg-d (95% CI 28.4-101.5) (Greene 2002b) is below the ADI. Only the upper confidence interval (101.5 μg/kg-d) begins to approach the ADI. If background exposure (23 μg/kg-d) is considered, the 99th percentile exposure (62.4 μg/kg-d) would be become 85.4 μg/kg-d), which is still below the ADI. Only the 95th upper confidence limit of the 99th percentile exposure would exceed the ADI if background exposure were included.

Working backwards, one may calculate the mouthing duration needed to reach the ADI value. Using average values for the product migration rate, *in vivo* and *in vitro* migration rates with the standard disk, and body weight, a 9-month-old child would have to mouth for approximately 13 hours per day to reach the ADI. Using the 95th percentile value for the product migration rate, and average values for all other parameters, the same child would have to mouth the object for 5 hours per day to reach the ADI. It appears that a mouthing duration of 5 hours (300 minutes) would only be attained at the 99th percentile value for pacifiers (≈370 minutes) (compare Greene 2002a, Table 10).

To summarize, the CPSC staff estimated oral exposures to DINP for various objects mouthed by children. The estimated exposures were dependent on the mouthing duration for each object category considered, and increased in the order: soft plastic toys < soft plastic toys, teethers, and rattles < all soft plastic objects < all toys, teethers, and rattles < pacifiers. For several object classes—ranging from "soft plastic toys" to "all toys, teethers, and rattles"—the mean and upper bound exposures were well below the ADI value of 120 μg/kg-d. This was true, even when the contributions from background dialkyl phthalate exposure were included.

Estimates of the oral exposure from pacifiers are higher than other products, but also are below the ADI value. For example, the estimated 99th percentile exposure in 3 to 12-month-olds of 62.4 μg/kg-d (95% CI 28.4-101.5) is below the ADI. Even the 95 percent upper confidence limit (101.5 μg/kg-d) of the 99th percentile value is below the ADI. However, considering background exposure (≈23 μg/kg-d), the 95% confidence interval of the 99th percentile exposure would include the ADI. However, it should be noted that confidence intervals have lower, as well as, upper limits. Therefore, the true 95th and 99th percentile exposures could be lower, as well as higher than, the best estimates.

In considering the estimated DINP exposures for the various object classes, it should be noted that all the migration data were obtained with soft plastic toys. However, less than half of the soft plastic toys tested contained phthalates (Chen 2002). Currently, teethers, rattles, and pacifiers sold in the U.S. do not contain phthalates. Not all soft plastic objects are made of PVC, and the category "all toys, teethers, and rattles" included materials other than plastic. Categories other than soft plastic toys were included as hypothetical examples of exposures that could result if the use of DINP were to increase in the future. The migration rates obtained with soft plastic toys would not necessarily be applicable to other products.

XI. Discussion

This risk assessment updates the 1998 CPSC staff risk assessment of oral DINP exposure from children's products (CPSC 1998). In 1998, the CPSC staff concluded that DINP exposure from soft plastic teethers and toys was below the ADI, but that there were significant sources of uncertainty in the exposure estimates, as well as in assessing the potential for carcinogenicity in humans. Since 1998, the CPSC staff convened a Chronic Hazard Advisory Panel (CHAP), conducted an observational study of children's mouthing behavior, participated in the development of a candidate standard method for measuring DINP migration, and obtained new DINP migration data.

A. Health Effects

In their report to the Commission, the CHAP discussed all the available data regarding the mechanisms by which peroxisome proliferators induce tumors in rodents, and their possible relevance to humans. This included a considerable amount of information that was published since 1998. The CHAP concluded that, although data in humans and other primates are limited, peroxisome proliferation is a process that is not easily induced in humans (CPSC 2001). The CHAP further concluded that it is unlikely that DINP could present a cancer hazard to humans under foreseeable conditions of exposure. The CPSC staff agrees that peroxisome proliferation is not readily induced in humans and, therefore, that DINP exposure is unlikely to present a cancer hazard to humans.

The liver is the most sensitive site for the non-cancer effects of DINP in animals. The CHAP concurred with the CPSC staff conclusion that the effects in the liver, including increased incidence of spongiosis hepatis, may be considered the critical endpoint for assessing the chronic health effects of DINP (CPSC 2001). The CHAP also agreed that the most sensitive study (i.e., Lington et al. 1997; NOAEL=15 mg/kg-d) should be used for setting an ADI, as in the 1998 CPSC risk assessment (CPSC 1998). Some authors have argued that a more recent study (Moore 1998a) with a higher NOAEL (88 mg/kg-d) should be used for setting an ADI. However, it became clear during the CHAP's deliberations that the disparate dose responses were due to methodological differences between the two studies (Babich and Greene 2000; EPL 1999; see also Brown 2000). Lington et al. examined at least four slides per liver, one from each lobe, whereas Moore examined only one slide per liver (EPL 1999). This difference in methodology is consistent with the more sensitive dose response seen by Lington et al. (1997).

Previously, the CPSC staff derived an ADI value of 150 μg/kg-d by applying a net uncertainty factor of 100 to the NOAEL (15 mg/kg-d) in the Lington et al. study (CPSC 1998). The CHAP also used the Lington et al. study, but employed a benchmark dose approach. They applied a net uncertainty factor of 100 to the estimated benchmark dose (D_{05}) of 12 mg/kg-d, resulting in an ADI of 120 μg/kg-d (CPSC 2001). Because both values were derived using accepted methods and neither estimate is clearly superior to the other, the lower value of 120 μg/kg-d was used in the present risk assessment.

The most recent European Union risk assessment used the NOAEL from the Moore (1998a) study to derive a tolerable daily intake (TDI) value (France 2000). However, in its review, the Scientific Committee on Toxicity, Ecotoxicity, and the Environment (CSTEE) of the European Commission agreed with the approach of the CHAP, which was to use a benchmark dose approach to derive an ADI from the Lington et al. study (CSTEE 2001).

The ADI is an estimate of the amount of chemical that a person can be exposed to on a daily basis over an extended period of time (up to lifetime) with a negligible risk of suffering deleterious effects. The ADI for DINP was derived from a lifetime dietary study in rats, and is 125-fold less than the NOAEL. This approach for deriving the ADI allows for the possibility that humans may be more sensitive than animals, but this is unknown for DINP. Therefore, exposure at levels slightly over the ADI or for less than a lifetime would not necessarily result in adverse health effects. Rather, it means that it cannot be assumed that the risk is negligible.

No data on the relative susceptibility of children or immature animals to DINP are available. In deriving ADI values for DINP, the CHAP (CPSC 2001) and the CPSC staff did not apply an additional uncertainty factor to account for the possibility that children may be more susceptible than adults. The lack of data on the effects of DINP in children or immature animals is a source of uncertainty in assessing the potential health effects of DINP (CPSC 2001).

DINP-A (71549-78-5) is a diisononyl phthalate that was never commercialized. The LOAEL in a two-year study in Sprague-Dawley rats was 27 mg/kg-d (Bio/dynamics 1986). This LOAEL was not used to derive an ADI, because DINP-A was never marketed (see part IV.D). However, DINP-A may be similar to the commercially available DINP-2. Hypothetically, if the study had been performed with a commercial DINP, the 27 mg/kg-d LOAEL could be used to calculate an ADI. The resulting ADI would be 27 µg/kg-d due to the additional uncertainty factor that is applied when a NOAEL has not been established (CPSC 1992). This would be roughly 5-fold lower than the current ADI. However, it should be noted that the incidence of necrosis at the mid dose in the DINP-A study (270 mg/kg-d) was not significantly different from the control (Table IV-4). Furthermore, use of a benchmark dose approach would result in a higher ADI, because an overall uncertainty factor of 100, rather than 1000, would be applied to the benchmark dose.

B. Oral Exposure and Risk

To estimate oral exposure to DINP in children's products, the CPSC staff measured DINP migration rates in 24 products (mostly soft plastic toys) by the JRC tumbling (i.e., "head-over-heels") method (Chen 2002). The DINP migration data were adjusted by an *in vivo*: *in vitro* ratio, using *in vivo* (RIVM 1998) and *in vitro* data with the PVC standard disk. The staff also conducted a new observational study of children's mouthing behavior (Greene 2002a; Kiss 2002). The CPSC study was similar in overall design to the study by Groot et al. (1998). However, the CPSC study differs in the use of a

demographically representative random sample, trained observers, larger sample size (n=169), and a more detailed classification of the objects mouthed.

Exposure estimates were derived for three age groups and several object classes. DINP was present in about 42 percent of soft plastic articles tested, but is not currently used in teethers and rattles (Chen 2002). Thus, we adjusted for the prevalence of DINP in calculating the exposure to soft plastic toys. This adjustment was only made for soft plastic toys (basic case). The basic case is the best estimate of current oral exposure to DINP in children's products. For all other exposure estimates, including a second calculation for soft plastic toys (hypothetical cases), the prevalence of DINP was assumed to be 100 percent. Hypothetical cases included: soft plastic toys; soft plastic toys, teethers, and rattles; all soft plastic objects; all toys, teethers, and rattles; and pacifiers. Hypothetical cases are the exposures that would result if DINP use in soft plastic toys and teethers were to increase. For example, in 1998 about 90 percent of soft plastic toys and teethers contained DINP. No pacifiers contained DINP, although a small number contained diisooctyl phthalate.

Distributions of the daily DINP exposures were estimated by Monte Carlo methods (bootstrap procedure), as described by Greene (2002b). Exposure estimates were derived by sampling from six distributions: migration data with 24 products, *in vitro* data with the standard disk, *in vivo* data with the standard disk, hourly mouthing time, exposure data (hours per day), and body weight. The procedure was implemented in a manner that preserved the dependence of the mouthing, weight and exposure on age, and the independence of the remaining variables. In the basic case (soft plastic toys, 42% containing DINP), 33 zero migration rates were added to the 24 non-zero rates. Exposure estimates were derived for the classes described above and three age groups (Table IX-9). Exposures for all object classes were based on the migration rates obtained for the same 24 children's products. The different exposures are primarily due to differences in mouthing duration for the different object classes and age groups and, in the basic case, to the difference in DINP prevalence. Because migration rates were obtained for soft plastic toys, but not teethers, rattles, or pacifiers, caution should be used in interpreting the results for objects other than soft plastic toys.

Exposures generally increased with age, although exposure for some objects was greatest in the middle (12-24 month old) age group (Table IX-9). Exposures generally increased in the order: soft plastic toys < soft plastic toys, teethers, and rattles < all soft plastic objects < all toys, teethers, and rattles < pacifiers. For all object classes, the resulting estimated exposures were lower than the ADI (120 μg/kg-d). For example, in the basic case (soft plastic toys, 42% containing DINP), the mean exposure among 12 to 24-month-olds was 0.08 μg/kg-d, with a 95th percentile of 0.53 μg/kg-d.

For the hypothetical case all toys, teethers, and rattles (100% containing DINP), exposure was greatest among 3 to 12-month-olds. The mean exposure was 2.9 μg/kg-d, while the median was 1.4 μg/kg-d. The 95th percentile exposure was 10.7 (95% CI 6.5-16.1) μg/kg-d.

The estimated exposure for the hypothetical case pacifiers was higher than for other products, due to the relatively long mouthing duration. However, all estimated exposures were below the ADI. Exposure was greatest among 3 to 12-month-olds, where the mean exposure was 4.8 μg/kg-d. The median was 0.00 (0.0-0.64) μg/kg-d, as 57 percent of children did not mouth pacifiers. The 95th percentile exposure was 24.6 (11.7-41.4) μg/kg-d (Table IX-9). The 99th percentile exposure (not shown in the Table) was 62.4 μg/kg-d (28.4-101.5) (Greene 2002b). Thus, even the 95% upper confidence limit of the 99th percentile exposure (101.5 μg/kg-d) is below the ADI of 120 μg/kg-d.

The low estimated exposure to DINP from soft plastic toys is consistent with a recent report in which MINP (a metabolite of DINP) was not detected in the urine of infants. Brock et al. (2002) measured monoalkyl phthalate levels in the urine of 19 infants ranging from 11 to 17 months of age. Samples were collected in early 2000, after manufacturers voluntarily removed DINP from teethers and rattles. Although other monoalkyl phthalates were detected, MINP was not detected in any of the samples tested.

The mean oral exposure estimates presented here are generally low in comparison to reported estimates of dermal exposure to DINP and background exposure to total dialkyl phthalates. For example, the dermal DINP exposure from handling soft plastic toys was estimated to be 1 μg/kg-d (France 2001), which is greater than the estimated mean oral exposures for soft plastic toys; soft plastic toys, teethers, and rattles; and all soft plastic items (compare Table IX-9). The CHAP estimated the dermal DINP exposure from rainwear and footwear to be 17 μg/kg-d for 19 to 36 month-olds (CPSC 2001), which is greater than the estimated 95th percentile oral exposures to all product classes, except pacifiers. The estimated background exposure to total phthalates was 23 μg/kg-d (Janssen et al. 1998). In comparing oral, dermal, and background exposures, it should be noted that different methods were used and limited data relating to dermal and background exposure were available.

If background exposure to other phthalates is considered, the total exposure could be increased by an estimated 23 μg/kg-d (Janssen et al. 1998). For most object classes, this would not increase exposures to the level of the ADI. The background exposures would be most significant for pacifiers, which have upper bound exposures closer to the ADI. If background exposure is considered, only the 95th upper confidence limit of the 99th percentile exposure for pacifiers (101.5 + 23=124.5 μg/kg-d) would exceed the ADI.

One source of uncertainty in the exposure assessment is the extrapolation of migration rates obtained with soft plastic toys to other product classes. Teethers, rattles, and pacifiers currently do not contain DINP. The other classes were included as hypothetical examples of the exposures that might occur if DINP were to be used in these products in the future. In the 1998 study, there did not appear to be any difference in migration rates between toys and teethers (Chen 1998a). Only one pacifier and one bottle nipple were tested, as most of these products were (and still are) made from latex or silicone. The migration rates for the pacifier and nipple, which contained diisooctyl phthalate, were relatively high when compared to toys and teethers containing DINP. Therefore, caution should be applied in interpreting the conclusions for products other than soft plastic toys.

Furthermore, certain toys comprising small or solid shapes were not amenable to testing by the JRC method.

Another source of uncertainty is in the composition of the objects mouthed in the observation study. The object class "all soft plastic items" included a large number of food contact items, such as cups, straws, and utensils, which are probably not PVC. The category "all toys, teethers, and rattles" included many items that were not made of plastic. This hypothetical class is included here because it is more comparable to the object classes in the Groot et al. study, and it is possible that the use of plastics such as PVC in these products could increase in the future. Therefore, the hypothetical exposure estimates presented here overestimate current exposures to DINP. However, the use of DINP and other phthalates in household products may change over time.

A third source of uncertainty is in the use of human subjects to validate the TNO and JRC migration methods. It is possible that some of the DINP migrating from the test materials in the human subject studies could have been absorbed through the oral mucosa (RIVM 1998). This could lead to underestimation of the *in vivo* migration rate. Some estimates suggest that the amount of DINP absorbed by this pathway is low (above, section IX.A.1.b; CPSC 2001, Appendix A). However, little is known about the relative permeability of the oral mucosa in comparison to other skin sites.

The exposure estimates presented here are generally lower than in previous reports. There are several reasons to explain this. Some risk assessments presented upper bound exposure estimates (e.g., CPSC 2001; France 2001). Several risk assessments used migration data from a small number of articles tested in human subjects (CPSC 2001; France 2001; Health Canada 1998; RIVM 1998), whereas both the present and earlier CPSC risk assessments used migration data from a range of children's products (CPSC 1998), and the basic case adjusts for the prevalence of DINP in soft plastic toys. The present risk assessment also uses an *in vitro*: *in vivo* ratio to adjust migration rates, which reduces the estimated mean exposure by about 50 percent.

However, the most significant difference among risk assessments is probably in the classification of objects mouthed by children. Some risk assessments have used more general object classes, such as "toys intended to be mouthed," "other toys," or "non-pacifiers." The CPSC observational study included more specific classes, such as "soft plastic toys," as well as more general classes, such as "all toys, teethers, and rattles." Classes such as "all toys" and "other toys" include objects that are not made of plastic. "Non-pacifier" predominantly includes anatomy (e.g., fingers and hands) and food contact items (e.g., cups and straws). Differences in study design between the CPSC observational study and previous studies may also be important, including the use of a demographically representative random sample, trained observers, relatively large sample size (n=169), and a detailed classification of the objects mouthed.

C. Dermal Exposure

Dermal exposure was not assessed in this report. For the products of interest—toys, teethers, and rattles—oral exposure from children's mouthing activity was the primary concern. There were no new data available, and in a previous risk assessment the relative contribution of dermal exposure was low in comparison to the oral exposure (CPSC 1983). DINP was absorbed slowly through the skin in animal studies (Stoltz and El-hawari 1983; Stoltz et al. 1985). The most recent European Union risk assessment estimated that the DINP exposure from handling soft plastic toys was about 1 μg/kg-d (France 2001). By one method, the CHAP estimated the exposures to DINP from rainwear and sandals in 19 to 26-month-old children to be 3.2 and 14 μg/kg-d, respectively (CPSC 2001 Appendix A), both of which are well below the ADI. Exposure estimates using a second method were much higher, but the CPSC staff considers the first method to be more appropriate (see above, part IX.B.2). The prevalence of DINP in PVC sandals and rainwear is unknown, but two pairs of sandals tested by CPSC contained both DEHP and dibutyl phthalate. Although most estimates of dermal DINP exposure were well below the ADI, they were higher than many of the oral exposure estimates presented here (compare Table IX-9).

The key input parameters needed to estimate dermal exposure are: the frequency and duration of contact with PVC products, the prevalence of DINP in these products, contact surface area, rate of DINP migration from PVC to skin, and rate of percutaneous absorption. Risk assessments made assumptions about the contact duration, as no data were available (CPSC 2001; France 2001). Reasonable estimates of the contact surface area can be made from published data on the surface areas of body parts, such as hands and arms (see EPA 1997). A study of the percutaneous absorption of DEHP from a PVC sheet in rats (Deisinger et al. 1998) was used as a surrogate for DINP (CPSC 2001; France 2001). This seems reasonable, because the rates of migration and absorption of DINP are not likely to be greater than those of DEHP and may be lower, based on their physico-chemical properties (CPSC 2001). Animal data on the percutaneous absorption of DINP are available (Stoltz and El-hawari 1983; Stoltz et al. 1985).

Sources of uncertainty in estimating dermal exposure include the lack of information on the frequency and duration of dermal contact and the prevalence of DINP or other phthalates in products other than toys. Another source of uncertainty is the effect of exposure conditions on the rate of migration and percutaneous absorption (CPSC 2001). For example, when wearing PVC sandals, the combination of pressure, friction, and moisture (perspiration) could increase both migration and percutaneous absorption. The extent to which these parameters would be affected is unknown.

D. Background Exposure

DINP represents about 15 percent of total dialkyl phthalate production (Madison et al. 2000). DINP has rarely been reported in food or the environment and, when present, it is at very low levels (CERHR 2000a). Other dialkyl phthalates—such as DEHP, dibutyl phthalate, and butyl benzyl phthalate—are ubiquitous environmental contaminants, found

in food and other environmental media, in addition to consumer products. Total phthalate exposure has been estimated to be 23 μg/kg-d in infants (Jannsen et al. 1998) and up to 30 μg/kg-d in the general population (references in Table IX-11).

Data on the health effects of combined exposures to dialkyl phthalates are not available. However, in the absence of data to the contrary, some risk assessors have assumed that the health effects of dialkyl phthalates are additive (CPSC 2001; RIVM 1998). Taking background exposure into account would not change any of the conclusions regarding oral exposure to DINP in children's products. Most of the oral exposure estimates would still be well below the ADI. The only exception is pacifiers, where the 99th percentile exposure would be close to the ADI. However, a risk assessment of the health effects from other phthalates or total phthalates is beyond the scope of this report.

E. Conclusions

- DINP is unlikely to present a cancer hazard to humans under foreseeable conditions of exposure.
- The acceptable daily intake level of 120 μg/kg-d derived by the CHAP, which is based on chronic health effects in the rat liver, is appropriate for assessing the chronic health effects of DINP in humans.
- No data are available regarding the relative sensitivity of children or immature animals to the health effects of DINP. CPSC does not apply an additional default uncertainty factor for children (CPSC 1992). The lack of data on the effects of DINP in children or immature animals is a source of uncertainty in assessing the potential health effects of DINP.
- Oral exposure to DINP from mouthing soft plastic toys is not likely to present a health hazard to children. All of the estimated average and upper bound exposures were below the acceptable daily intake value of 120 μg/kg-d.
- If DINP were to be used in soft plastic teethers and rattles, oral exposure to DINP from mouthing these products probably would not present a health hazard to children. This conclusion is based, in part, on migration rates obtained with soft plastic toys. Currently, teethers and rattles are not made with DINP or other dialkyl phthalates.
- If DINP were to be used in pacifiers, the oral exposure to DINP probably would not present a health hazard to children. If background exposure is considered, the 99th percentile exposure (and its statistical upper confidence limit) would be close to the ADI. This conclusion is based, in part, on migration rates obtained with soft plastic toys. Currently, pacifiers do not contain DINP or other dialkyl phthalates. Most pacifiers are made of either latex or silicone.
- One source of uncertainty in assessing oral exposure to DINP is the amount that may be absorbed through the oral mucosa. Information on the relative permeability of the oral mucosa to DINP or other hydrophobic substances would reduce this source of uncertainty.

- Dialkyl phthalates are ubiquitous environmental contaminants. No data are available on the combined effects of phthalates, many of which induce similar health effects. If it is assumed that the health effects from total phthalate exposure are additive and of similar potency to DINP, then the estimated exposures from toys, teethers, and rattles would still be below the acceptable daily intake. However, an assessment of the possible health effects of exposure to other phthalates or total phthalates is beyond the scope of this report.

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